

EPIDEMIOLOGY OF RHODOCOCCLUS (CORYNEBACTERIUM) EQUI IN FECAL
AND ENVIRONMENTAL SAMPLES FROM KANSAS HORSES AND LOCATIONS

by

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B.S., Kansas State University, 1979

D.V.M., Kansas State University, 1979

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

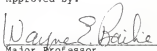
MASTER OF SCIENCE

Department of Laboratory Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1986

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DEDICATION

To my husband, Brad

ACKNOWLEDGMENTS

Words cannot express my gratitude to Dr. Wayne Bailie, my major professor, for his cooperation and guidance throughout this endeavor. Completion of this thesis would have been impossible without his effort and encouragement.

I also appreciate the opportunity given by the Department of Laboratory Medicine and the assistance of the Department of Laboratory Diagnosis which allowed completion of graduate studies. I wish to thank all persons in the Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, who provided me with assistance toward completion of this thesis.

TABLE OF CONTENTS

	Page
Dedication	i
Acknowledgments	ii
Introduction	1
Review of the Literature	3
Materials and Methods	34
Results	50
Discussion	79
Literature Cited	98
Appendix	115

INTRODUCTION

Rhodococcus (Corynebacterium) equi was first discovered and described by Magnusson in Sweden in 1923. At nearly the same time, Bull isolated the organism in southern Australia and later published a description (Bull, 1924). Both Magnusson and Bull considered infections produced by the organism to be a new disease. Magnusson called the disease "Specific Contagious Pneumonia in the Foal" and suggested the name Corynebacterium equi for the organism (Magnusson, 1923). Earlier workers had observed R. equi infections in lung abscesses of foals, but considered the organism a contaminating saprophyte because other bacteria were frequently recovered from the same lesions (Magnusson, 1923, 1938).

Following these first reports, the bacterium was recovered from animal infections in many other countries (Barton and Hughes, 1980). Dimock and Edwards recognized the first case of the infection in the United States in Kentucky in 1931. They observed that the disease had an insidious onset and usually affected foals less than 6 months of age. Some farms had more death losses than others and the incidence varied from year to year.

Rhodococcus equi is the etiological agent of a sporadic pneumonia of foals. The disease is highly fatal because lesions in the lungs become severe before clinical signs are observed. Occasionally the organism may cause

extrapulmonary abscesses in horses of any age. Abscesses in immunosuppressed humans have been reported to be caused by the organism (Barton and Hughes, 1980).

The epidemiology of a disease is an important consideration when designing a control program. At this time, little is known about this aspect of this disease and more knowledge is needed for adequate development of such programs (Report of the Foal Pneumonia Panel, 1978).

Previous work established that a number of different capsular serotypes of R. equi exist. The purpose of this study was to determine if the serotypes of R. equi found in the environment or in the equine digestive tract are the same as those found in infections of foals.

The specific objectives of this study were: (1) To examine various cultural techniques for recovery of R. equi from contaminated sources such as soil and feces. (2) To compare the rate of recovery of R. equi from soil and feces on farms with and without a history of infections in foals. (3) To serotype all R. equi recovered and compare fecal and environmental isolates with isolates from foals with disease. (4) To utilize the serotyping information to trace the origin of infection in foals and to develop management procedures to prevent R. equi foal pneumonia.

REVIEW OF THE LITERATURE

History

Following the first descriptions of R. equi induced foal pneumonia by Magnusson (1923) and Bull (1924), isolation of the organism from cases of the disease was reported by workers throughout the world (Table 1). Many different names have been used for the organism since it was first described. The synonyms and their authors are presented in Table 2. At present, both Corynebacterium equi and Rhodococcus equi are listed as legitimate names in the Approved List of Bacterial Names (Skerman et al., 1980).

Taxonomy

Taxonomic studies conducted by a variety of researchers determined that R. equi, then classified as C. equi, should be moved to a different genus although few agreed which genus was correct (Stuart and Pease, 1972; Goodfellow and Alderson, 1977; Reddy and Kao, 1978; Collins et al., 1982 and Barton and Hughes, 1982). Several reports finally classified the organism in the genus Rhodococcus as a soil associated actinomycete (Goodfellow et al., 1976; Goodfellow and Alderson, 1977 and Barton and Hughes, 1982). However, some numerical taxonomic studies failed to separate R. equi from the genus Corynebacterium (Barksdale, 1978). Since recognition of R. equi as a name for C. equi, Goodfellow et al. (1982) reported "while chemical, genetic and numerical phenetic studies favor the recognition of Rhodococcus equi,

Table 1. Reports of Recovery of Rhodococcus equi from Cases of Foal Pneumonia

Country	Author/s	Date
Scandinavia	Flatala	1942
	Ek and Nordstoga	1967
Australia	Wilson	1955
	Bain	1963
		1969
	Dewes	1972
	Roberts and Polley	1977
	Gay <u>et al.</u>	1981
Germany	Miessner and Wetzel	1923
	Lutje	1923
	Lind	1939
Great Britian and Ireland	Farrelly	1951
	Guvén	1963
	Linton and Gallaher	1969
	Platt	1973
India	Rajogaplan	1937
Japan	Harakawa and Morita	1949
Russia	Reshetnyak	1942
Yugoslavia	Sterk and Sebetic	1956
South Africa	Grosskopf <u>et al.</u>	1957
Argentina	Campero <u>et al.</u>	1981
United States		
Kentucky	Dimock and Edwards	1931
		1932
	Rooney	1966
California	Britton	1945
	Smith and Robinson	1981
Washington State	Burrows	1968
New York State	Shively <u>et al.</u>	1973
Alabama	Bowman	1977
Minnesota	Meunier and Rings	1975

Table 2. Synonyms for Rhodococcus equi.

Synonym	Author	Date
<i>Corynebacterium equi</i>	Magnusson	1923
<i>Corynebacterium pyogenes</i> (<i>equi</i>)	Miessner and Watzel	1923a 1923b
<i>Corynebacterium pyogenes equi</i> (<i>roseum</i>)	Lutje	1923
<i>Corynebacterium pyogenes</i>	Lund Witt	1924 1933
<i>Mycobacterium equi</i>	Jensen Krasil'nikov	1934 1966
Holth's <i>Bacillus</i>	Bendixen and Jepsen Jespersen Holth and Amundsen Plum	1940 1938 1936 1940
<i>Bacterium viscosum equi</i>	Dimock	1939
<i>Corynebacterium Magnusson</i> Holth	Plum Ottosen	1940 1941
<i>Corynebacterium purulentus</i>	Holtman	1945
<i>Mycobacterium rhodocrous</i>	Gordon	1966
<i>Rhodococcus equi</i>	Goodfellow and Alderson	1977

they are not sufficiently comprehensive to characterize the species or determine its homogeneity".

Lysogenic strains of R. equi exist (Bica-Popii et al., 1969). One study determined that all isolates examined were lysogenized with bacteriophages (Meitert et al., 1975 cited by Barton and Hughes, 1980).

Rhodococcus equi possesses a capsule which is non-toxic and unaffected by hyaluronidase (Smith, 1966). "The adsorption of capsular substance to erythrocytes, its staining with alcian blue [which has a strong affinity for polysaccharide capsules of bacteria] and its resistance to trypsin, indicate [the] polysaccharide nature" (Carter and Hylton, 1974). Electron microscopy of ruthenium red stained mounts revealed that the capsule had a wavy, laminated appearance (Woolcock and Mutimer, 1978). Ruthenium red stains polysaccharide.

Pili, 4-6 nm in diameter, and often exceeding the bacterial cell in length, have been found on 10-37 % of cells cultivated on ordinary nutrient agar (Yanagawa and Honda, 1976). The role of pili in the pathogenesis of infections has not been determined.

Pradip et al. (1966b) reported that the pigments of R. equi were carotenoids which required thiamine for synthesis, because thiamine induced the enzyme system required for carotenoid synthesis. Consequently, pigment production may well be correlated with the nature of the medium on which

isolates were studied (Barton and Hughes, 1980).

Arabinose and galactose are the primary cell wall sugars of R. equi and strains of mycobacteria (Cummins, 1962) which share common antigens. The similarity of cell wall composition and antigenic structure may partly explain why tuberculosis-like granulomas are produced in R. equi infected cattle.

Rhodococcus equi cell membranes contain menaquinones which function in electron transport and oxidative phosphorylation. The main polar lipids are glycolipids and phospholipids.

Fraser (1964) first reported a diffusible substance produced by R. equi which potentiated the effect of staphylococcal beta-hemolysin, a reaction which is similar to that produced by the action of Lancefield group B streptococci on the same staphylococcal hemolysin and known as the CAMP reaction (Christie et al., 1944). In addition, he found synergistic hemolysis when R. equi was grown with Corynebacterium pseudotuberculosis and Listeria monocytogenes. Combination of phospholipase-C of R. equi with phospholipase-D of C. pseudotuberculosis and magnesium ions caused a synergistic hemolysis of erythrocytes, especially ruminant erythrocytes. Phospholipase-C, an extracellular protein of R. equi, is capable of hydrolyzing all of the isolated major phospholipids of mammalian erythrocyte membranes (Bernheimer et al., 1980).

Since Fraser's finding that a culture of R. equi was CAMP positive, other investigators have tested their isolates in this reaction. Barton and Hughes (1982) found 94 % of their isolates were positive to the test. Prescott et al. (1982) found 100 % of their isolates were positive and coined the term "equi factor" for the substance produced by R. equi which enhanced the staphylococcal hemolysin.

Goodfellow and Alderson (1977) proposed placing the former C. equi into the genus Rhodococcus in the family Nocardiaceae based on the following criteria: (1) Cell wall composition: a. "Menaquinone composition distinguished the... representatives of the 'rhodocrous complex'... from mycobacteria" (Collins et al., 1977). b. Arabinose and galactose are contained in the cell wall of NCTC 1621 (Keddie and Cure, 1977). (2) Mycolic acids: The mycolates of R. equi are larger than the mycolic acids found in animal associated coryneforms (Collins et al., 1976). (3) Aerobic growth: An early report of anaerobic growth (Magnusson, 1923) was probably due to poor anaerobic conditions (Barton and Hughes, 1982). (4) Existence of a fragmentary vegetative mycelium.

Media and Biochemicals

When R. equi was first cultured, it was grown on common laboratory media such as nutrient agar, potatoes, egg yolk, and Loeffler's serum (Magnusson, 1923 and Bull, 1924).

Investigators later noted that it demonstrated moist, runny colonies, or dry growth similar to tubercle bacilli, depending on the medium (Verge and Senthille, 1942b). They also noted that it produced more pigment on some media than others (Dimock, 1931).

Jensen (1934) first used tellurite blood agar (the medium then used for routine inoculation of Corynebacterium diphtheriae for isolation of R. equi. Others followed in the use of tellurite in selective media, because R. equi colonies were black when they reduced the tellurite in the medium (Rajagopalan, 1937; Wilson, 1955; Woolcock et al., 1979; Woolcock et al., 1980 and Barton and Hughes, 1981a). Barton and Hughes (1981a) added tellurite to M-3 agar medium (Rowbotham and Cross, 1977) to aid in the identification of R. equi colonies and coined the term M3T medium. This medium was useful for isolation of R. equi from contaminated samples because it was not rich enough to support the growth of many contaminants and contained cycloheximide as a further inhibiting factor. Barton and Hughes (1981a) also utilized NANAT-tellurite agar medium (Woolcock et al., 1979) which was a richer medium than M3T, but contained more inhibitors such as nalidixic acid, novobiocin and actidione (cycloheximide). The very rich chocolate blood agar medium described by Smith and Robinson (1981) for recovery of R. equi from soil and fecal samples, contained polymyxin B and amphotericin B as inhibitors, and tellurite as an aid

in identification of R. equi colonies. Another procedure which enhanced recovery of R. equi from contaminated sources consisted of subculturing from a selective broth onto a selective agar containing one or more of the same inhibitors found in the broth. This approach was successful in numerous studies (Woolcock et al., 1980; Barton and Hughes, 1981a and Smith and Robinson, 1981). Most investigators reported the production of a salmon pink to brick red pigment by R. equi when grown on various media (Magnusson, 1923; Bull, 1924; Karlson et al., 1943; Woodruff, 1950; Linton and Gallaher, 1969; Goodfellow and Alderson, 1977 and Barton and Hughes, 1982). However, other workers reported tan pigment (Bruner and Edwards, 1941 and Holtman, 1945), fawn pigment (Rajagopalan, 1937), yellow pigment (Brooks, 1942), yellow-tan pigment (Dimock and Edwards, 1931 and Dennis and Bamford, 1966), orange pigment (Karlson and Thoen, 1971) and non-pigmented strains (Magnusson, 1938; Rahman, 1957 and Jang et al., 1975).

Colonies are non-hemolytic (Magnusson, 1923; Rajagopalan, 1937; Weaver et al., 1974; Jang et al., 1975). Isolation of one hemolytic strain was reported (Dafaala et al., 1961 cited by Barton and Hughes, 1980).

Because of the mucoid nature of the growth, several investigators demonstrated the presence of capsule using the India ink method (Dimock and Edwards, 1932; Bruner and Edwards, 1941 and Karlson, et al., 1943). A colorless area

around the bacterial cell delineated by India ink was suggestive of a capsule. However, other researchers failed to demonstrate a capsule (Rajagopalan and Gopalakrishnan, 1938). Karlson et al. (1943) demonstrated that the treatment which destroyed the serologic type specificity (which was considered to be located in the capsule) did not alter the results of the India ink treatment. They concluded that "the evidence obtained is inadequate to indicate that the type specificity and the mucoid growth of our culture of C. equi are due to a demonstrable capsular material". The organism is now considered to be encapsulated and recent work demonstrated that growing R. equi on Mueller-Hinton agar encouraged capsule production (Prescott, 1981a).

Several investigators have reported colonial variants which are not mucoid, but more discrete and opaque (Karlson et al., 1943; Craig and Davies, 1940; Woodruff, 1950 and Roberts, 1957). The pathogenicity of these "Type D" colony variants is in question (Mutimer and Woolcock, 1981).

The organisms usually appear as Gram-positive cocci or pleomorphic rods (Magnusson, 1923; Bull, 1924; Weaver et al., 1974 and Barton and Hughes, 1982). Gram-negative forms may appear in older cultures (Cotchin, 1943).

Some workers demonstrated that the organism was acid-fast (Holth and Amundsen, 1936; Rajagopalan, 1937 and Gordon, 1966). However, others have not been able to

demonstrate the acid-fastness (Rajagopalan, 1937; McCarter et al., 1939; Holtman, 1945 and Karlson and Thoen, 1971). It has been suggested that acid-fastness possibly depends on the age of the culture, older cultures becoming acid-fast positive (Bendixen and Jepsen, 1940 and Verge and Senthille, 1942b).

Rhodococcus equi is catalase positive (Weaver et al., 1974; Mutimer and Woolcock, 1980 and Barton and Hughes, 1982), and non-motile (Bull, 1924; Weaver et al., 1974; Berg et al., 1977; Gardner et al., 1976 and Barton and Hughes, 1982), even though Magnusson (1923) decided his culture was motile.

Although R. equi is relatively inactive biochemically, most strains hydrolyze urea and reduce nitrates to nitrites (Mutimer and Woolcock, 1980; Prescott, 1981a and Barton and Hughes, 1982). However, urea and nitrate negative strains have been described (Natajara and Nilakantan, 1974; Nakazawa, 1980 and Barton and Hughes, 1982).

Other differential tests such as indole, casein, gelatin, methyl-red, Voges Proskauer, esculin and litmus milk are usually negative (Weaver et al., 1974; Mutimer and Woolcock, 1980; Barton and Hughes, 1982). Indole positive (Rahman, 1957) and esculin positive (Prescott, 1981a) strains have been reported. Reports on the production of hydrogen sulfide by R. equi are conflicting. This appears to be due to the use of different detection media by various

investigators (Mutimer and Woolcock, 1981).

Growth on MacConkey agar has been reported (Rajagopalan, 1937 and Barton and Hughes, 1982). Others failed to obtain growth on this medium (Gardner et al., 1976; Addo and Dennis, 1977 and McKenzie and Donald, 1979). This may be due to the varied ability of strains to grow in the presence of 0.01 % crystal violet (Barton and Hughes, 1982).

Most investigators agree that R. equi does not oxidize or ferment glucose or other carbohydrates (Magnusson, 1923; Bull, 1924; Weaver et al., 1974 and Barton and Hughes, 1982). Gordon (1966) reported that the type strain NCTC 1621 produced acid from glucose oxidatively, while Davis and Newton (1969) stated that this strain fermented this substrate. These results have not been successfully repeated.

Although R. equi does not appear to produce acid from glucose, the presence of the carbohydrate in the medium will stimulate growth of the organism (Pradip et al., 1966a). It is likely that glucose is metabolized to acetate which is stimulatory to growth of R. equi (Pradip et al., 1966b).

Resistance

The marked resistance of R. equi to a variety of chemical compounds was first noticed when investigators were using these treatments on tuberculous lymph nodes of swine and cattle to aid in the isolation of mycobacteria. The

organism was resistant to 2-5 % oxalic acid for 60 minutes (Karlson et al., 1943 and Linton and Gallaher, 1969), to 15 % sulfuric acid for 45 minutes (Cotchin, 1943), to 0.0001 % crystal violet and 0.01 % sodium azide for at least 24 hours (Goodfellow and Alderson, 1977) and to full strength "chlorox" for at least 10 minutes (Holtman, 1945). Sodium hydroxide has been used as a selective digestant for recovery of R. equi from animal tissues (McKenzie and Donald, 1979) but Karlson et al. (1943) demonstrated that agar slant cultures were 99.5 % killed by a 0.5 % concentration of this chemical in 15 minutes.

Rhodococcus equi is killed at a temperature of 58 C in 20 minutes (Sippel et al., 1968). It is also susceptible to homogenization (Bendixen and Jepsen, 1938) and 1% phenol in 10 minutes (Holtman, 1945). The organism could still be recovered from inoculated soil samples after one year of exposure to sunlight (Wilson, 1955 and Smith and Robinson, 1981).

Conflicting reports of the sensitivity of R. equi to various antimicrobial agents are numerous. Such controversy may be due to strain variation, differences in testing methods or observations that treatment of infected animals was not successful.

Resistance to penicillin is reported both by failure of this agent to successfully treat infected animals and by in vitro susceptibility tests (Linton and Gallaher, 1969;

Meunier and Rings, 1975; Barton and Hughes, 1980 and Goodfellow et al., 1982). However, Prescott (1981b) reported that intravenously administered penicillin was an effective treatment.

Erythromycin, kanamycin, gentamycin and sulfonamides appear consistently effective in in vitro tests (Meunier and Rings, 1975; Knight and Heitala, 1978; Prescott, 1981b and Smith, 1982). However, Barton and Hughes (1982) reported that sulfadiazine was not effective in their in vitro tests.

Susceptibility to tetracyclines, streptomycin, neomycin, chloramphenicol, ampicillin and other penicillin derivatives appears to vary depending upon the strain of R. equi tested (Linton and Gallaher, 1969; Marsh and von Graevenitz, 1973; Knight and Heitala, 1978 and Barton and Hughes, 1982). The organism is usually susceptible to neomycin, streptomycin, and chloramphenicol, but is usually resistant to penicillin derivatives and is unpredictable with tetracyclines.

Antigenic Structure

Because R. equi was relatively inactive in differential test media, early workers attempted to utilize serology to help distinguish the organism from other "Chromogenic diphtheroids" (Magnusson, 1940; Dimock and Edwards, 1931; Feldman et al., 1940 and Karlson et al., 1943). Methods used were adapted from procedures followed for dealing with Corynebacterium pyogenes (Brown and Orcutt, 1920) and

Corynebacterium diphtheriae (Robinson and Peeny, 1936).

With continued study, two different types of antigens were revealed: (1) type or group specific and (2) species specific antigens. The type specific antigens were superficially located and were assumed to be a part of the capsule of the bacterium (Bruner et al., 1939; Karlson et al., 1943; Woodruff, 1950 and Prescott, 1981a). It is generally accepted that the type specific antigen is located in the capsule (Prescott, 1981a). This antigen diffuses into broth or saline when untreated cells are suspended in these liquids (Prescott, 1981a and Mutimer et al., 1982). Prescott (1981a) identified seven different capsular serotypes and standardized the nomenclature.

The second antigen revealed was species specific. It was exposed with hot acid treatment of cells which extracted the type specific antigens from the surface of the bacterium (Magnusson, 1938; Bruner et al., 1939; Karlson et al., 1943 and Woodruff, 1950). The vigorous treatment required to expose the species specific antigen indicated that it was part of the somatic structure of the bacterium and not an exposed determinant (Knight, 1969). Antigen preparations used by researchers for serologic tests depended on which determinants the individual desired to detect, i.e. the type specific or the species specific antigens.

Type specific antigens

The simplest type-specific preparation was a 10%

suspension of whole untreated organisms and was used in an agglutination reaction (Karlson et al., 1943; Bruner and Edwards, 1941 and Woodruff, 1950). Supernatants of broth cultures were used by others for precipitin tests (Carter and Hylton, 1974; Prescott et al., 1979 and Prescott, 1981a). The supernate from the hot acid extraction procedure (used to obtain species specific antigen) was also used as a type specific antigen for precipitin tests (Bruner and Edwards, 1941; Cotchin, 1943 and Woodruff, 1950). Later, the supernatants of broth or saline suspensions were treated with trypsin and adsorbed with immune sera (Carter and Hylton, 1974), dialyzed against distilled water (Prescott et al., 1979) or precipitated with ammonium sulfate, dissolved and then dialyzed (Nakazawa, 1980).

Species specific antigens

Species specific antigens, which are probably located in the cell wall, distinguish R. equi from other closely related species and genera (Woodruff, 1950). A hot acid treatment removed the type specific antigen from the cells and after centrifugation, the supernate was discarded and the remaining cells used in a complement fixation test (Bruner and Edwards, 1941; Karlson et al., 1943; Cotchin, 1943 and Woodruff, 1950). Since that time, less emphasis has been placed on the detection of the species specific antigens (Prescott, 1981a).

Production of antiserum

Repeated injections of rabbits with either killed or live cultures of R. equi have been used to stimulate production of satisfactory antisera (Bruner et al., 1939; Feldman et al., 1940; Woodruff, 1950; Carter and Hylton, 1974; Smith and Robinson, 1981 and Prescott, 1981a). However, others have been unable to produce acceptable antisera with such procedures (Magnusson, 1923 and Cotchin, 1943). The reason/s for this discrepancy are unknown.

Prescott (1981a) successfully produced rabbit antiserum for capsular serotyping. Cultures incubated at 37 C for 48 hrs were washed from Mueller Hinton agar with 0.15 M saline. The live bacterial suspensions were given intravenously every three to four days incrementing "from 0.5 ml by 0.5 ml to a maximum of 3 ml." The eighth or ninth inoculation consisted of 1.5 ml of a R. equi suspension and 1.5 ml Freund's complete adjuvant which was "injected subcutaneously and intramuscularly in equal parts into the rabbits." If precipitin lines were absent after this inoculation schedule, two to three more intravenous inoculations of 0.5 ml live R. equi suspension induced precipitins in the sera. Prescott (1981a) used a double diffusion precipitin test. A plastic template which contained one central and six peripheral wells was placed on the surface of 1 % Noble agar. The central well was filled with antisera and the peripheral wells with appropriate

antigen preparations.

Production of antisera in other animal species has met with mixed success. Efforts to produce agglutinins in horses by repeated intravenous injections were successful for Dimock and Edwards (1931) and Magnusson (1938) but not for Rajagopalan (1937). Cotchin (1943) was unable to produce precipitins in swine.

Studies to detect agglutinins in normal horses, in spontaneously affected animals and mares with fertility problems were not successful (Bull, 1924; Magnusson, 1938 and Nakazawa, 1980). More sensitive techniques such as the fluorescent antibody technique and the in vitro anti-hemolysin potentiation test have detected antibodies against R. equi in human, horse and donkey serum (Wilks, 1981 and Seddick, 1982).

Epidemiology

The epidemiology and habitat of R. equi are controversial subjects. It has been recovered from a variety of different types of soils (Wilson, 1955; Smith and Robinson, 1981; Woolcock et al., 1980 and Barton and Hughes, 1982). The organism appears to be short-lived in some soils. It is recovered at a low rate from neutral, alkaline, clay-based and wet soils (Jones, 1975; Smith and Robinson, 1981; Barton and Hughes, 1981 and Robinson, 1982).

Some investigators believe there is no evidence that the habitat is in the soil (Mahaffey, 1962; Woolcock et al.,

1979 and Woolcock and Mutimer, 1980/1981). However, most persons agree that soil is an important source of infection for foals (Magnusson, 1938; Report of the Foal Pneumonia Panel, 1978 and Robinson, 1982).

Foals which are born in late winter or early spring are less frequently infected than those born in the late spring or summer (Magnusson, 1938). It has been suggested that this may be related to increased dust during the summer in many areas (Smith, 1982 and Robinson, 1982). Even though R. equi is present in areas never inhabited by horses, it is found in greater numbers where horses have been housed (Woolcock et al., 1980; Barton and Hughes, 1981b; Smith and Robinson, 1981 and Robinson, 1982). Barton and Hughes (1981b) concluded that R. equi counts in feces increased ten-thousand fold three weeks after collection of the specimen and remained at a high level for an additional two weeks. This finding strengthens the idea that R. equi may live in soil without feces, but numbers may be higher if horses, and consequently horse feces, are present (Magnusson, 1938; Woolcock et al., 1980; Robinson, 1982 and Smith, 1982). However, a clinical impression of Linton and Gallaher (1969) was that horses did not appear to carry the organism from infected to non-infected premises.

Rhodococcus equi is probably maintained in the mare and immunologically competent foal as a normal non-invasive member of the flora of the gastrointestinal tract (Robinson,

1982). Wilks (1981) provided evidence that horses respond to this floral carrier state with an immunologic response. Eighty percent of horses on premises with and without a history of recurrent R. equi pneumonia in foals were positive on both the skin and indirect fluorescent antibody tests. Continual or intermittent shedding of the organism from feces supplemented the soil reservoir (Barton and Hughes, 1981b; Robinson, 1982 and Smith, 1982). Furthermore, infected feces may serve as a source of infection to foals during the first 4-5 weeks of life when they commonly ingest fresh feces from the dam (Urquhart, 1981).

The organism also appears to reside in the gastrointestinal tract of cattle and sheep. Woolcock and Mutimer (1980/1981) examined specimens at five different levels of the tract and found R. equi in one or more locations from 88 % of the animals. Cattle do not appear to respond to the carrier state with a humoral immune response (Seddick, 1982). However, McKenzie and Ward (1981) concluded that infections in cattle cause false positive results in the complement fixation test for Johne's disease. They concluded that R. equi possessed antigens in common with Mycobacterium paratuberculosis. They also stated that R. equi infections were unlikely to produce false positive intradermal tuberculin reactions in the field.

Rhodococcus equi also produces abscesses in a low percentage of swine submaxillary and cervical lymph nodes.

Jespersen (1938b) reported that 8 % of all tuberculosis condemnations were actually due to R. equi infections. Cultures isolated from lymph nodes of swine and pneumonic lungs of foals had the same species specific antigens (Rajagopalan and Gopalakrishnan, 1938; Bruner et al., 1939; Karlson et al., 1943 and Woodruff, 1950). Prescott (1981a) demonstrated that there was no clear relationship between a given type-specific antigen and the origin of the isolate.

Incidence

Rhodococcus equi induced foal pneumonia is most common in foals 2-4 months of age, but they may be less than one month or as old as seven months at the onset of the disease (Dimock and Edwards, 1931; Rajagopalan, 1937; Reshetnyak, 1940; Bain, 1963 and Campero et al., 1981). The presence of the disease on a farm usually causes serious financial loss because it is often fatal. Reported foal mortality rates due to the infection were under 25 % (Reshetnyak, 1940), between 40 and 70 % (Bain, 1963; Sippel et al., 1968 and Campero et al., 1981) or as high as 80 % (Magnusson, 1923).

Miessner and Koser (1931) listed R. equi as the fifth most common isolate from foal cadavers in Germany. In a 10 year study, Farrelly (1969) estimated that the organism caused 2-3 % of all foal infections in Great Britain and Ireland. Platt (1973), also in Great Britain, reported 3 of 61 generalized bacterial infections of foals were due to R. equi. Although statistics were not given, the Report of the

Foal Pneumonia Panel (1978) stated: "the prevalence of C. equi (R. equi) in the equine population seems to be increasing in certain areas of the country" (United States of America).

Of forty infected foals necropsied, Hutchins et al., (1988) reported that 65 % were Thoroughbreds, 27.5 % were Arabians and 7.5 % were Standardbreds. Wilks et al. (1982) suggested that these figures may not reflect a particular breed susceptibility, but might be a reflection of overall numbers in the population.

Laboratory animals

Because animal inoculation is a useful method for studying and identifying bacteria, many early investigators studied the pathogenicity of R. equi in a variety of species of laboratory animals (Bull, 1924; Dimock and Edwards, 1931; Rajagopalan, 1937; Magnusson, 1938; Bruner and Edwards, 1941; Karlson et al., 1943 and Pullin, 1946). Results obtained were extremely variable. Pathogenicity appeared to vary from one report to another. This variability may have been due to R. equi strain variation, differences between strains of laboratory animals of the same species or individual animal variation. The route of inoculation appeared to be a factor in the pathogenicity of this organism in laboratory animals. The most recent study of the organism's pathogenicity in laboratory animals was conducted by Mutimer and Woolcock (1982b). Intravenously

challenged mice tolerated up to 100 million cells in their liver without apparent systemic involvement or fatality. Ten million cells administered either orally or subcutaneously also resulted in no apparent systemic signs. Intranasal administration of 10 million but not 1 million cells produced a pneumonia with a slow clearance of the organism from the lungs.

Clinical signs

Based on duration of clinical signs, acute, subacute and chronic forms of R. equi infection in foals were described. The acute form usually affected foals less than one month old, and death occurred 24-48 hours after onset of clinical signs. With the subacute form, the foals lived 1-2 weeks after the first appearance of clinical signs (Wilson, 1955; Mahaffey, 1962; Rooney, 1966). Foals with the chronic form of the disease displayed dullness and increased respiratory rate, which slowly progressed over weeks to dyspnea, coughing and moist rales. Although foals with this form continued to suckle until death, they became weak and emaciated (Hughes, 1966; Bredin, 1973). Moist rales were a common feature of all three forms, but purulent nasal discharge was an inconsistent sign (Mahaffey, 1962; Bain, 1963; Bredin, 1973).

Acute fulminating diarrhea caused by R. equi was described in foals with and without R. equi pneumonia (Merritt et al., 1975; Rooney, 1966; Hutchins et al., 1980).

If a foal developed watery green, foul-smelling diarrhea, indicating the enteric form of disease, the prognosis was poor (Rooney, 1966).

Diseases of foals other than bronchopneumonia and enteritis

Rhodococcus equi infections in foals are not limited to the more common pneumonic and enteric forms. Abscesses of vascular, subcutaneous, and lymphatic tissue, as well as infections of bones and joints have been reported in foals under one year old with no signs of pneumonia or of enteritis (Bain, 1963; Platt, 1973 and Smith, 1980). However cellulitis, and infections of the kidneys, eyes, joints, bones, pleural cavity and vascular tissues are more commonly reported in conjunction with enteritis or pulmonary abscesses (Magnusson, 1938; Britton, 1945; Bain, 1963 and Bowman, 1977).

Diseases in adult horses

Adult horses are occasionally infected by R. equi. Descriptions of bronchopneumonia and pulmonary abscesses, abortions, infertility, liver involvement, lymphangitis, arthritis, nephritis, mastitis, enteritis and subcutaneous abscesses have been reported from all over the world (Carne, 1927; de Lacerda and Viega, 1959; Simpson, 1964; Merritt et al., 1975 and Genetsky, 1982).

Pathology-Histopathology

Rhodococcus equi causes a suppurative bronchopneumonia with multiple pulmonary abscesses. Abscesses of various

sizes were sometimes slightly raised above the normal pleural surface of the lung (Dimock and Edwards, 1931; Mahaffey, 1962 and Roberts and Polley, 1977). Considerable fibrosis of affected lung tissue suggested long duration of the disease regardless of duration of clinical signs (Wilson, 1955 and Bain, 1963). Hutchins et al. (1980) proposed that microscopic abscessation and ulceration of the intestinal tract might be detectable in most foals even when not grossly apparent. Abscessation of regional lymph nodes and adjacent organs, such as the liver, has been described (Dimock and Edwards, 1931 and Bain et al., 1969).

Pathogenesis

Rhodococcus equi is either inhaled or ingested before it establishes an infection in the foal. While both routes of infection are possible, the respiratory route is probably more common. Either route of infection may be followed by lymphatic or hematogenous spread of the organism (Linton and Gallaher, 1969; Urquhart, 1981 and Smith, 1982). The polysaccharide capsule may inhibit phagocytosis and prevent antibody production, although a humoral response may not be important for protection against this disease (Smith, 1966 and Hutchins et al., 1980). Host response to infection includes fibrosis and an acute suppurative process, commonly resulting in multiple abscessation throughout the lungs (Wilson, 1955, Mahaffey, 1962 and Burrows, 1968).

Apparently, R. equi is pathogenic only when there is

massive challenge and/or when the host's immune system is not capable of responding sufficiently (Wilks, 1981). The organism may escape normal pulmonary defense mechanisms by intracellular survival (Martens, 1982). Since R. equi is present in the intestines of a high percentage of horses, minor intestinal invasion without typical disease probably occurs in the early life of most horses (Prescott et al., 1980).

Immunity

Carter and Hylton (1974) first suggested that immunity to R. equi was more cell-mediated than antibody-mediated. Clumps of bacteria persisted within the cytoplasm of macrophages in R. equi lesions for up to 90 days after inoculation of the organisms into the animal (McKenzie, Donald and Dimmock, 1981). Subsequently, Martens (1982) proved that the bacterium was an intracellular parasite within macrophages. He observed that extracellular R. equi organisms had apparently been released from dead or dying phagocytes.

Association with parasites and streptococci

Association of R. equi infections with parasitism has been reported (Burrows, 1968; Bradin, 1973 and Campero, 1981). When an adequate parasite control program was initiated, R. equi foal pneumonia disappeared from studs in Australia where the disease had previously been endemic. Sporadic cases again appeared when parasite control was

relaxed (Bain, 1963). Bain et al. (1969) discovered Habronema sp. larval stages associated with R. equi abscesses in the lung of a foal. Dewes (1972) correlated high Strongyloides westeri egg counts with R. equi abscessation of inguinal and axillary lymph nodes of foals. He surmised that Strongyloides westeri-induced skin damage preceded infection by R. equi from contaminated soil or feces.

Streptococci have often been present in R. equi lung abscesses (Mahaffey, 1962; Hughes, 1966 and Smith, 1982). An outbreak of strangles was accompanied by an unusually high mortality rate due to secondary invasion of the lungs by R. equi (Hughes, 1966 and Rooney, 1966).

Prevention-Control

Recommended management procedures for prevention or control of R. equi infections either: (1) lowered the total number of organisms to which the foal was exposed, or (2) enhanced maintenance of optimum foal health (Bain, 1963; Smith, 1982 and Wilks et al. 1982). Movement of mares to "clean" foaling pastures, control of parasites, vaccination for viral diseases and reduced aerosol exposure to dust were all successful management procedures (Magnusson, 1938; Bain, 1963 and Smith, 1982). Isolation of sick foals and vigilant surveillance for early signs of disease were also recommended even though the disease is not significantly contagious (Mahaffey, 1962 and Smith, 1982). Vaccination

with R. equi bacterins was not uniformly successful (Anonymous 1, 1982).

Treatment

Because R. equi causes the formation of multiple thick walled abscesses within the lungs of affected foals, treatment of the disease has been effective only if initiated early and then continued for days or weeks (Barton and Fulton, 1980; Gay et al., 1981 and Smith, 1982). Various antimicrobials or combinations thereof were inconsistently effective in treatment of R. equi infections (Shively et al., 1973; Meunier and Rings, 1975; Merritt, 1975 and Smith and Robinson, 1981). Campero et al. (1981) observed that antimicrobials only delay the terminal phase of the disease.

Merritt (1975) suggested that certain antibiotics were effective in some geographic areas but not in others, because R. equi antimicrobial sensitivities varied. However, Larson (1980) proposed that R. equi became resistant to certain antimicrobials as the disease progressed.

Diseases in other species

A number of reports detailed the isolation of R. equi from species other than the horse. Isolations from swine were predominantly associated with concurrent or suspected tuberculous infections of the submaxillary and cervical lymph nodes (Barton and Hughes, 1980). Reported disease in

ruminants ranged from pneumonia and lymphadenitis to reproductive tract infections (Table 3) (McKenzie and Donald, 1979; Lloyd and Peet, 1979; Woolcock and Mutimer, 1980/81; Rogers et al., 1980; Barton and Hughes, 1980 and Singh, 1982). Isolation of R. equi from cat abscesses and from a variety of diseases in exotic species has been documented (Stein and Stott, 1979; Barton and Hughes, 1980 and Higgins and Paradis, 1980). Immunosuppressed humans have developed pulmonary abscesses and pneumonia (Table 4) (Berg et al., 1977; Mutimer et al., 1979 and Barton and Hughes, 1980).

Table 3. Reports of Rhodococcus equi Isolations from Ruminants.

Syndrome	Country	Author
<u>Cattle</u>		
Pyometra	United Kingdom	Craig and Davis (1940)
Chronic pneumonia	United States	Holtman (1945)
Ulcerative lymphangitis	Kenya	Neave (1951)
Mesenteric lymph node abscess	Denmark	Jorgensen (1966)
Pneumonia	India	Moitra (1972)
Bronchial and mesenteric lymph node abscess	Australia	Woolcock and Rudduck (1973)
Mastitis, vaginitis, metritis	India	Natarajan and Nilakantan (1974)
Lymphadenitis	Australia	McKenzie and Donald (1979)
Lymphadenitis normal feces	Australia	Lloyd and Peet (1979)
normal feces	Australia	Woolcock and Mutimer (1980/81)
lymphadenitis	Australia	Mutimer and Woolcock (1980)
<u>Buffalo</u>	Australia	Rogers et al. (1980)
vaginitis	India	Rajagopalan and Gopalakrishnan (1938)
pneumonia	India	Singh (1982)

Table 3 (continued)

Syndrome	Country	Author
<u>Goat</u>		
pneumonia and liver abscesses	United States	Whitford and Jones (1974)
abscesses	India	Natarajan and Nilakantan (1974)
pneumonia and arthritis	Australia	Barton (unpublished)
<u>Sheep</u>		
chronic pneumonia	Australia	Roberts (1957)
abortion	Australia	Dennis and Bamford (1966)
CNS lesion	Australia	Dennis and Bamford (1966)
bronchopneumonia	Nigeria	Addo and Dennis (1977)
normal feces	Australia	Woolcock and Mutimer (1988)

Table 4. Reports of Rhodococcus equi Isolations from Species Other Than Horses, Pigs or Ruminants.

Syndrome	Country	Author
Cat lymphadenitis pyothorax, pulmonary abscess abscess abscess	United States Australia Australia Canada	Jang <u>et al.</u> (1975) Barton and Hughes (1988) Barton and Hughes (1988) Higgins and Paradis (1988)
Koala rhinitis and pneumonia	Australia	Rahman (1957)
Cottontop marmoset thoracic abscess	United States	Stein and Stott (1979)
Alligator and Crocodile fulminating bacteremia	United States	Jasmin <u>et al.</u> (1969)
Humans pulmonary abscess pulmonary abscess pulmonary abscess	United States United States United States	Golub <u>et al.</u> (1967) Williams <u>et al.</u> (1971) Marsh and von Graevenitz (1973)
necrotizing pneumonia pneumonia pulmonary abscess pulmonary abscess and bacteremia	United States United States Australia	Carpenter and Blom (1976) Gardner <u>et al.</u> (1976) Savdie <u>et al.</u> (1977)
weight loss, fever, diarrhea	United States Australia	Berg <u>et al.</u> (1977) Mutimer <u>et al.</u> (1979)

MATERIALS AND METHODS

Selection of Locations for Specimen Collection

Farms selected for collection of specimens to be cultured were chosen in consultation with attending veterinarians on the staff of the Department of Surgery and Medicine, College of Veterinary Medicine, Kansas State University. Four different farms were selected from which to collect specimens. They were designated: 1) Control Farm^a, 2) Infected Farm I^b, 3) Infected Farm II^c, and 4) Previously Infected Farm^d. Selection of the Control Farm which had no history of past clinical infections with R. equi was based on clinical observations. Infected Farm I and Infected Farm II were detected when animals submitted to the Kansas State University Veterinary Hospital yielded either ante- or post-mortem positive cultures of the organism in the Clinical Bacteriology Laboratory or the Department of Veterinary Diagnosis, Kansas State University. The Previously Infected Farm was chosen when the attending veterinarian^e of Infected Farm I communicated information in regard to this farm to the investigator. Additionally, specimens were collected from the environment in the Kansas State University Veterinary Hospital and from horses which were routinely admitted as patients.

^a Alta Vista, Kansas

^b Valley Falls, Kansas

^c Chapman, Kansas

^d Tonganoxie, Kansas

^e Dr. Linda Gratney, Leavenworth, Kansas

Collection of Fecal Specimens

Fresh fecal specimens were collected from each horse on every farm. Fresh was defined as less than six hours old. The majority of the specimens were collected rectally in a disposable plastic obstetrical sleeve/glove^a. Following collection of the sample from the rectum of a horse, the sleeve was immediately everted with the feces inside of the glove portion. Each specimen was identified and placed on ice. A history was recorded which included age, sex, breed, date, current administration of therapeutic agents, length of time at the location and diet. Additionally, disease status of the horse or its progeny was recorded. When it was impossible to collect a specimen directly from the rectum of the horse, as with young foals or fractious stallions, freshly defecated specimens were used. When such specimens were collected from the ground or a floor, contamination with dust, dirt and bedding was avoided as much as possible. Specimens were maintained at 4-5 C and processed within 24-36 hours of collection. No adult horse from which a specimen was collected had an apparent gastrointestinal infection.

Statistical Analysis

Chi-square analysis was used to examine the significance of differences in R. equi isolation rates (Snedecor and Cochran, 1980). A probability (P value) of a Kane Enterprises, Ag-Tek Division, Sioux Falls, S.D.

0.05 or less was interpreted as a significant difference.

Collection of Soil Samples

One or more soil samples of at least 5 gm each were collected from each lot, paddock or pasture on each of the farms. The location in the area from which each sample was to be collected was determined by as random a method as possible. Two different methods were used. Either a different person entered an adjacent area and selected the location or a sampling stick was thrown backward over the shoulder, and the sample was collected where the stick fell. In all instances, locations which were heavily contaminated with horse feces were avoided. No effort was made to choose a particular type of soil. Samples were picked and stored in clean plastic covered specimen containers and stored in the dark at 25 C until processed.

Collection of Other Environmental Samples

Environmental samples collected from stalls inside of barns were cobwebs since the floors of most of the facilities were heavily contaminated with horse feces. Cobwebs also collect environmental dust and should represent a sampling of the soil in the facility. Samples were collected at least four feet above the level of the floor. Samples were variable in weight due to availability and were delivered to the laboratory in clean plastic covered containers. They were stored in the dark at 25 C until processed. In the Kansas State University Veterinary

Hospital, where cobwebs are not allowed to accumulate, dust from overhead air circulation filters was collected.

Collection of Bird Feces

Feces expressed from the cloacae of a chicken and a baby sparrow from a nest in a barn were collected on Infected Farm I. These feces were handled as were equine feces.

Selective Media

A total of six different types of selective broth and agar media were utilized either singly or in combination in attempts to recover R. equi from the specimens. All media contained one or more substances which selectively inhibited growth of some species of bacteria or fungi. Compositions, names and abbreviations of these media are presented in Table 5. See Figure 1 for details of the isolation scheme. Approximately 1 gm of soil or feces and a variable amount of cobweb was inoculated into the broth media. Broths were incubated under aerobic conditions at 37 C and subcultured onto selective agar media at 4 and 7 days post-inoculation. When broth media were subcultured to agar plates, a sterile swab was inserted into the broth medium, excess fluid expressed on the side of the tube and the swab used to inoculate the initial area on the plate. Agar media in 15 x 100 mm plastic disposable petri dishes^a were inoculated with approximately 0.3 gm of specimen and streaked for isolation.

^a Fisher Scientific Co., St. Louis, MO.

Table 5: Composition of Selective Media.

A. Trypticase Soy Broth, Cycloheximide, Nalidixic Acid, Penicillin Broth "TANP", (Barton and Hughes, 1981)

Trypticase Soy Broth (a)	30.0	gm
Cycloheximide (b)	0.05	gm
Nalidixic Acid (b)	0.02	gm
Penicillin-G (b)	0.01	gm
Potassium Tellurite (b)	0.05	gm
Distilled Water	1000	ml

B. Brain Heart Infusion Broth with Polymyxin-B, "BHI-6", (Smith and Robinson, 1981)

Brain Heart Infusion (c)	37.0	gm
Polymyxin-B (b)	0.006	gm
Distilled Water	1000	ml

C. Brain Heart Infusion Broth with Polymyxin-B and Amphotericin-B, "BHI-12+2" (Smith and Robinson, 1981)

Brain Heart Infusion (c)	37.0	gm
Polymyxin-B (b)	0.012	gm
Amphotericin-B (b)	0.002	gm
Distilled Water	1000	ml

D. Nalidixic Acid, Novobiocin, Cycloheximide, Potassium Tellurite Agar, "NANAT" (Woolcock, et al., 1979)

Tryptic Soy Broth (c)	30.0	gm
Yeast Extract (c)	1.0	gm
Bacto-Agar (c)	15.0	gm
Nalidixic Acid (b)	0.02	gm
Novobiocin (b)	0.025	gm
Potassium Tellurite (b)	0.05	gm
Cycloheximide (b)	0.04	gm
Distilled Water	1000	ml

E. Dextrose Proteose # 3 Medium with Polymyxin-B, Amphotericin-B, Potassium Tellurite and Chocolatized Bovine Blood, "Dex-Prot" (Smith and Robinson, 1981)

Dextrose Proteose # 3 Agar (c)	40.0	gm
Polymyxin-B (b)	0.012	gm
Amphotericin-B (b)	0.002	gm
Bovine Blood (added at 80 C)	50	ml
Distilled Water	1000	ml

Table 5 (continued)

F. M 3 Agar Medium (Rowbotham and Cross, 1977)

Potassium Phosphate (monobasic) (f)	0.466	gm
Sodium Phosphate (dibasic) (d)	0.732	gm
Potassium Nitrate (e)	0.1	gm
Sodium Chloride (e)	0.29	gm
Magnesium Sulfate . 7 H ₂ O (d)	0.1	gm
Calcium Carbonate (e)	0.02	gm
Sodium Propionate (b)	0.2	gm
Ferrous Sulfate . 7 H ₂ O (g)	0.2	gm
Zinc Sulfate . 7 H ₂ O (e)	0.18	gm
Manganese Sulfate . 7 H ₂ O (d)	0.015	gm
Bacto-Agar (c)	18.0	gm
Cycloheximide (b)	0.05	gm
Thiamine (b)	0.004	gm
Distilled Water	1000	ml

G. M 3 T Agar Medium (Barton and Hughes, 1981)

M 3 Agar Medium plus		
Potassium Tellurite (b)	0.05	gm

a = BBL, Div. Becton, Dickinson, and Co.,
Cockeysville, MD.

b = Sigma Chemical Co., St Louis, MO.

c = Difco Laboratories, Detroit, MI.

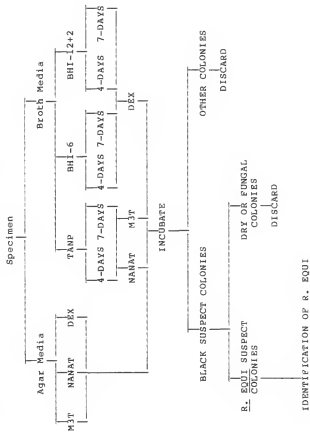
d = Fisher Scientific Co., St Louis, MO.

e = Mallinckrodt Chemical Works, St Louis, MO.

f = J. T. Baker Chemical Co., Phillipsburg, NJ.

g = Specialty Chemicals Division, Morristown, NJ.

Figure 1. Selective Media Inoculation Scheme



KEY TO ABBREVIATIONS:

- M3T = M3 MEDIUM OF ROWBOTHAM AND CROSS (1977) PLUS POTASSIUM
TELLURITE (BARTON AND HUGHES, 1981)
- NANAT = NALIOXIC ACID, NOVOBIOICIN, CYCLOHEXIMIOE, POTASSIUM
TELLURITE MEDIUM (WOOLCOCK ET AL., 1979)
- OEX = OEXTROSE PROTEOSE # 3 AGAR, POLYMYXIN-B,
AMPHOTERICIN-B, POTASSIUM TELLURITE, STERILE
CHOCOLATIZED BOVINE BLOOD (SMITH AND ROBINSON, 1981)
- TANP = TRYPTICASE SOY BROTH, CYCLOHEXIMIOE, NALIOXIC ACID,
PENICILLIN (BARTON AND HUGHES, 1981)
- BHI-6 = BRAIN HEART INFUSION BROTH, POLYMYXIN-B (SMITH AND
ROBINSON, 1981)
- BHI-12+2 = BRAIN HEART INFUSION BROTH, POLYMYXIN-B,
AMPHOTERICIN-B (SMITH AND ROBINSON, 1981)

Agar media were incubated under aerobic conditions at 37 C for 7 days and then stored at room temperature in sealed plastic bags for an additional 14 days. Plates were examined at weekly intervals for the presence of colonies of R. equi. When typical or suspect colonies were observed, they were subcultured to blood agar (BA) (Tryptic Soy Agar^a plus 5 % sterile bovine blood) for isolation and identification.

Identification of Isolates

Colonies which were dark colored or black and moist on selective agar media were subcultured to BA plates, incubated aerobically at 37 C for 24-48 hours, Gram stained, tested for catalase production and then subjected to the CAMP test (Christie et al., 1944; Fraser, 1964 and Prescott et al., 1982). One to three isolated colonies of each CAMP positive strain were inoculated into differential media using standard procedures. Differential media used were Triple Sugar Iron Agar (TSI)^a, Christensen's Urea Agar^b, Esculin Broth composed of Heart Infusion Broth^a containing 0.1 % Esculin^c, Infusion Agar Slants (Heart Infusion Broth^a plus 1.5 % Bacto-Agar^a), Oxidation-Fermentation Medium (Bacto-Agar^a 0.3 %, Peptone^a 0.5 %, Glucose^c 1.0 % and Phenol Red^c 0.003 % pH 7.3) and Casein Agar (Tryptic Soy Agar^a containing 5 % powdered skim milk).

Inoculated media were incubated under aerobic

a Difco Laboratories, Detroit, Mich.

b Baltimore Biological Laboratories, Cockeysville, Md.

c Sigma Chemical Company, St. Louis, Mo.

conditions at 37 C for 7 days and results recorded using accepted procedures (Buchanan and Gibbons, 1974 and Weaver et al., 1974). Colony morphology and pigmentation were recorded after 24-48 hours incubation at 37 C on BA. Cultures identified as R. equi were preserved by freezing at -60 C on glass beads (Nagel and Kunz, 1972).

Antiserum Production

Antisera against the 7 described serotypes of R. equi (Prescott, 1981a) were produced in rabbits obtained through the Animal Resource Facility, Kansas State University. Three rabbits were immunized with each serotype. Stock cultures of the 7 serotypes were obtained from Dr. J. F. Prescott^a and maintained frozen at -60 C on glass beads (Nagel and Kunz, 1972).

Suspensions of live R. equi of each serotype to be used as antigen were prepared as described by Prescott (1981a) and enumerated by the pour plate method as described by Koch (1981). The first injection for all serotypes was with live bacterial suspensions emulsified in equal parts of Freund's Incomplete Adjuvant^b. Subsequent injections were with unadjuvantized, live bacterial suspensions. Rabbits were first inoculated subcutaneously with 1.0 ml of the immunogen in 5-7 separate areas over their back and 0.5 ml injected into the heavy musculature of each hind leg. Subsequent

^a University of Guelph, Guelph, Ontario, Canada

^b Difco Laboratories, Detroit, Mich.

immunizations were in 0.5 ml amounts and injected into the musculature of each hind leg. Rabbits were injected 6 times at approximately 3 week intervals. Rabbits were bled prior to immunization, 5-7 days after each inoculation and at the end of the series. Blood was allowed to clot, the serum separated, and stored in labeled glass tubes at -10 C.

Capsular Antigen Preparation

Capsular antigen was extracted from R. equi cells of the 7 described serotypes for immunodiffusion, counterimmunoelectrophoresis and adsorption of antisera. Extraction in early studies was by a minor modification of the method described by Prescott (1981a). Briefly, a lawn of growth was washed from the surface of a Mueller-Hinton Agar^a plate which contained an additional 1 % Bacto-Agar^a with 5 ml of 0.15 M NaCl, the suspension was incubated at 37 C overnight and then centrifuged. The clear supernate which served as the antigen, was preserved with 0.34 % Sodium Azide^b and stored in sterile screw capped glass test tubes at 4 C. These preparations were not highly satisfactory and an alternative method was used later in the study.

Capsular Antigen Preparation - Alternative Method

Each serotype of R. equi was inoculated onto 28-48 Mueller-Hinton Agar^a plates containing 1 % additional Bacto-Agar^a to obtain nearly confluent growth. Plates were

^a Difco Laboratories, Detroit, Mich.

^b Sigma Chemical Co., St. Louis, Mo.

incubated at 37 C for 48 hours and the growth harvested with a rubber policeman while the petri dish was rotated on a "Spray-Fisher" Turntable^a. Bacterial cells were suspended in sterile 0.15 M NaCl, 3 ml per plate of growth harvested, in a sterile screw capped 250 ml ehrlenmeyer flask. The suspension was gently shaken in a 37 C water bath for 2 hours, transferred to a sterile 50 ml centrifuge tube and centrifuged at 30877 x g for 20 minutes. The supernate was removed with a sterile Pasteur pipette and repeatedly re-centrifuged until a clear, colorless supernate was obtained. The solution was dialized for 48 hours against three changes of 0.005 M Tris-HCl^b buffer pH 8.5 containing 0.04 % Sodium Azide^b. Antigen was stored in sterile screw capped test tubes at 4 C.

Antiserum Adsorption with Whole Bacterial Cells

Mueller-Hinton Agar^c containing 1 % additional agar in sterile disposable 15 x 100 mm petri-dishes^a was inoculated to prepare a confluent lawn of growth. Plates were incubated aerobically at 37 C for 48 hours. Growth was harvested with a rubber policeman while the petri-dish was rotated on a "Spray-Fisher" Turntable^a. Bacterial cells were mixed with the appropriate antiserum to be adsorbed at a rate of cells from 2.5 plates to 1 ml of serum. The mixture was incubated in a 50 C water bath for 2-4 hours and

^a Fisher Scientific Co., St. Louis, Mo.

^b Sigma Chemical Co., St. Louis, Mo.

^c Difco Laboratories, Detroit, Mich.

shaken every 15 minutes. Following incubation the mixture was centrifuged at $27138 \times g$ for 25 minutes to sediment the cells. Serum was removed and in some instances re-adsorbed up to four times using the same procedures. Adsorbed serum was preserved by the addition of sodium azide^a 0.05 % and stored at -10 C.

Adsorption of Antiserum with DEAE/Capsular Antigen

The appropriate antiserum was precipitated with 50 % saturated ammonium sulfate using standard methods (Garvey, Cremer and Sussdorf, 1977). The resulting precipitate of crude immunoglobulin was re-dissolved in distilled water to the original volume of antiserum. This solution was repeatedly dialyzed against 0.15 M NaCl until sulfate ions were not detected in the dialyzate. Antibody preparations were then dialyzed against 0.005 M Tris HCl^a buffer pH 8.6 (Tris).

Capsular antigen, prepared by the alternative method as described above, was adsorbed to DEAE Bio-Gel A^b (DEAE-A). DEAE-A was equilibrated to pH 8.6 with three changes of Tris. Prior to binding antigen to DEAE-A, the optimal binding pH was determined by mixing antigen and DEAE-A at a variety of pH's and then testing the supernate for antigen. The pH at which antigen could not be detected in the supernate was selected as the optimum binding pH. Antigen

^a Sigma Chemical Co., St. Louis, Mo.

was added to the DEAE-A and allowed to adsorb by constant stirring on a laboratory rotator for 30 minutes. To insure complete saturation of the DEAE-A, four changes of antigen were allowed to adsorb. Following the adsorption, the mixture was gently centrifuged and the supernate removed. To adsorb an antiserum, crude immunoglobulin was diluted with an equal volume of Tris^a, one ml of the diluted serum was mixed with one ml of the antigen coated DEAE-A^b and the suspension mixed on a clinical rotator for 30 minutes. Following the adsorption period, the suspension was lightly centrifuged and the antiserum decanted. Adsorbed antiserum was stored in glass tubes at -10 C.

Agar Gel Double Immunodiffusion Precipitation

Precipitation tests were conducted in 1 % Agarose (Type I, Low EEO)^a. Two mm thick slabs of agarose were prepared on GelBond Film^c according to the manufacturers instructions (Anon. 2, 1982). Briefly, the hydrophobic side of an 82 x 135 mm sheet of GelBond film was adhered to a 82 x 101 mm glass plate with a few drops of distilled water. A 2 mm thick U shaped plexiglass spacer with 10 mm wide sides was placed on the hydrophilic side of the film. A second 82 x 101 mm glass plate was placed on the spacer and the casting unit clamped together with two binder clips on each side. The unit was supported vertically on a level surface by the

a Sigma Chemical Co., St. Louis, Mo.

b Bio-Rad Laboratories, Richmond, Calif.

c FMC Corporation, Rockland, Maine

binder clips and agarose dissolved in 0.15 M NaCl and cooled to 55 C was pipetted into the unit. After the agarose had solidified, the glass plates and spacer were removed. A central well and six peripheral wells were cut into the resulting 2 x 65 x 91 mm agar slab attached to the film. Wells were 2 mm in diameter and placed 5 mm equidistant from each other. Agarose plugs were removed with a Pasteur pipette attached by a rubber hose to a water vacuum. Each agarose slab accommodated six to eight sets of wells.

Wells were carefully filled with approximately 60 microliters of the appropriate antiserum (central well) or antigen (peripheral wells) and incubated in a moist chamber at room temperature. Bands of precipitation were detected utilizing indirect fluorescent light and recorded at 24, 48 and 72 hours incubation. Following the last observation, gels were washed, pressed, and dried according to the manufacturers instructions (Anon. 2, 1982). The dried agarose gel was then stained with 0.2 % Coomassie Brilliant Blue R-250^a in methanol: glacial acetic acid: water (5:1:5, v/v/v), destained in the same solvent and air dried.

Counterimmunoelectrophoresis

Precipitin tests were also conducted in agarose gels by counterimmunoelectrophoresis utilizing methods described by Crowle (1980). Briefly, 1 % Agarose, Type III, High EEO agarose^a was melted in 0.5 M Barbitol Buffer^a. Two mm thick a Sigma Chemical Co., St Louis, Mo.

agarose slabs were prepared on GelBond Film^a in the same casting unit as described for gel diffusion. Two rows of seven pairs of wells each were cut into the agarose slab utilizing a laboratory fabricated jig. Wells were 2 mm in diameter and wells of each pair were spaced 10 mm apart. Appropriate crude immunoglobulins or antisera were placed in the wells on the anodic side and antigen on the cathodic side. Electrophoresis was conducted utilizing 0.5 M Barbitol Buffer^b at a constant current of 20 ma for 1 hour utilizing a regulated direct current power supply^c. Following electrophoresis, agarose slabs were washed, pressed, dried and stained as was described for gel diffusion and the precipitin bands recorded.

^a FMC Corp. Rockland, Maine

^b Sigma Chemical Co., St. Louis, Mo.

^c Duostat, Beckman Instruments Inc., Palo Alto, California

RESULTS

Collection and Culture Results of Fecal SpecimensControl Farm

Fecal material from a total of 26 horses was sampled on the Control farm. Seventeen of these were Quarter Horses and 9 were Thoroughbreds. Rhodococcus equi was recovered from 8 of these samples. Four isolates were obtained from each of the breeds. There was no significant difference in the proportion of isolations between the breeds. A summary of results with regard to sex, age and breed of the horses on this farm is presented in Table 6. Other details of information about these horses may be found in appendix Tables 1 and 2.

Infected Farm I

All horses on this farm were Arabian or at least 3/4 Arabian blood. This farm was the only one on which samples were collected on two different occasions. On the first sampling, 7 fecal specimens were collected. Two of these samples yielded cultures of R. equi. On the second sampling, 27 fecal specimens were collected. Two of these fecal specimens were positive for R. equi. Both horses whose feces yielded the organism were stallions. Two foals and 4 adults were sampled both the first and second times. Feces from one horse (K-27) was positive on the first sampling but not the second. One of the horses, a male foal (K-8-A), was positive on the first sampling, but died of a

Table 6. Results of Culture of Horses by Sex, Age and Breed.

Horses Sampled	Control Farm	Infected Farm I	Infected Farm II	Previous Farm	KSU Hospital	All Locations
	8/26 ^a	(A) 2/7 (B) 2/27	2/9	15/24	17/21	46/114
Sex of Horses						
Female	7/22	(A) 1/5 (B) 0/17	2/6	15/21	7/11	32/82
Male	1/4	(A) 1/2 (B) 2/10	0/3	0/3	9/9	13/31
Unknown	0	0	0	0	1/1	1/1
Age of Horses						
0-6 months	0/6	(A) 2/3 (B) 0/3	1/4	0	2/3	5/19
> 6 months to 1 year	3/8	(A) 0 (B) 0/3	0	1/2	1/2	5/15
> 1 year to 3 years	0/1	(A) 0 (B) 1/10	0	1/3	2/3	4/17

> 3 years	5/11	(A) 0/4 (B) 1/11	1/5	13/19	11/12	31/62
Unknown	0	0	0	0	1/1	1/1
Breed of Horses						
Thoroughbred	4/9	0	0	0	6/7	10/16
Quarter Horse	4/17	0	2/3	15/24	8/10	29/54
Arabian	0	(A) 2/7 (B) 2/27	0	0	1/1	5/35
Paint	0	0	0/6	0	0	0/6
Clydesdale	0	0	0	0	1/1	1/1
Appaloosa	0	0	0	0	0/1	0/1
Unknown	0	0	0	0	1/1	1/1

a = Number Positive / Number Sampled
 (A) = First Sampling
 (B) = Second Sampling

confirmed R. equi infection prior to the second sampling. A summary of results with regard to sex, age and breed of the horses on this farm is presented in Table 6. Other details of information about these horses may be found in appendix Tables 1 and 2.

Infected Farm II

Animals sampled on this farm were Paints (6) or Quarter Horses (3). A total of 2 of these fecal samples were positive for R. equi. One of the samples was not collected at the farm, but at the KSU Veterinary Hospital after the foal (D-9) had been submitted for an illness. The sample was collected from the foal at necropsy. A summary of results with regard to sex, age and breed of the horses on this farm is presented in Table 6. Other details of information about these horses may be found in appendix Tables 1 and 2.

Previously Infected Farm

All animals on this farm were Quarter Horses between the ages of 1 and 32 years. Fecal specimens were collected from 24 horses and of these, 15 were positive for R. equi. A summary of results with regard to sex, age and breed of the horses on this farm is presented in Table 6. Other details of information about these horses may be found in appendix Tables 1 and 2.

Hospitalized Horses

A total of 21 horses representing 5 different breeds

were sampled at the KSU Veterinary Hospital. Fecal specimens from all except 4 of the horses yielded cultures of R. equi. A summary of results with regard to sex, age and breed of the horses sampled in this location is presented in Table 6. Other details of information about these horses may be found in appendix Tables 1 and 2.

Summary of all Horses

A total of 114 horses representing 6 different breeds were sampled from the 5 locations. Forty-six (40.4 %) of these fecal specimens yielded cultures of R. equi. A summary of results with regard to sex, age and breed of the horses sampled and positive in these locations is presented in Table 6.

Statistical Evaluation of Cultural Results

The data obtained failed to reveal a significant difference in the isolation rate from feces between the two infected farms or between the two infected farms and the Control Farm. However, there was a significantly greater proportion of isolations on the Previously Infected Farm than on the Control Farm ($p = 0.025$) and on the infected farms ($p = 0.000$). Likewise, there was also a greater proportion ($p = 0.000$) of isolations from hospitalized horses when compared to non-hospitalized.

Demonstration of statistical differences in isolation rates in regard to ration, time at farm and breed were not successful. However, horses over three years of age had

higher isolation rates than horses three years of age or younger ($p = 0.025$).

Collection and Culture Results of Soil Samples

Control Farm

Six soil samples were collected from 5 different paddocks and a pasture. A culture which was definitely R. equi was recovered from one of these samples (H-S-5). An additional soil sample (H-S-6) yielded a culture which was similar to R. equi in appearance, but was urea and nitrate negative and esculin positive. This sample was taken from a paddock in which there were no horses at the time of sampling. Since this isolate was not entirely typical of R. equi, it was not considered as this species.

Infected Farm I

Ten soil samples were collected at this farm. Nine samples were from different paddocks in which horses were kept and 1 sample from a clean stall. Three of these samples (K-S-5, K-S-10 and K-S-11) which were collected from paddocks were positive for R. equi. No culture positive horses were detected in the paddocks from which these samples were collected.

Infected Farm II

Nineteen soil samples were collected from 9 different paddocks and 2 pastures. One of these samples (D-S-20) was positive for R. equi and it was collected from an alfalfa pasture which did not contain any horses at the time of

sampling but had previously been used.

Previously Infected Farm

Twelve soil samples were collected from 5 different paddocks and 4 pastures. Two of these samples yielded cultures of R. equi. One of these samples (C-S-19) was collected from a pasture which contained four horses all of which had R. equi in their feces. The other sample (C-S-16) was collected from a paddock which was at present unused, but often used to hold breeding mares.

Summary of all Farms

A total of 47 soil samples were collected from 28 paddocks, 7 pastures and one stall on all of the locations. Seven (14.9 %) of these samples yielded cultures of R. equi. Five (10.6 %) of these positive samples were collected from paddocks and two (4.3 %) from pastures. The sample collected from the stall was not culture positive.

Collection and Culture Results of Other Environmental Samples Control Farm

Two samples of cobwebs were collected from 2 different barns. One of the samples yielded a culture of R. equi. It was collected from a building in a paddock which contained 2 culture positive horses.

Infected Farm I

Three samples of cobwebs were collected from 3 different stalls in one barn. One of these samples was culture positive. This sample was from a stall in which 2

horses were housed. Rhodococcus equi was not recovered from fecal samples from these horses.

Infected Farm II

Fifteen samples of cobwebs were taken from 4 different barns. None of these samples yielded cultures of R. equi.

Previously Infected Farm

Cobweb samples were taken from 4 different buildings. None of the 13 samples collected were positive for R. equi.

Summary of all Farms

Thirty-three samples of cobwebs were collected from 11 buildings on the farms. Two (6.1 %) of these specimens yielded cultures of R. equi.

Hospital

Dust accumulated in ceiling filters above the equine stalls of the KSU Veterinary Hospital was collected from 12 different filters. None of these samples yielded R. equi.

Collection of Bird Feces

Infected Farm I

Two fecal samples were collected from birds in one of the buildings. One sample was collected from a baby sparrow which was in a nest and the other was from a chicken. The feces was expressed from the cloacae into a clean container. Both of these samples yielded R. equi on culture. There were 9 stalls in this building and 7 horses were in the stalls. One of these horses (K-4B) was positive. Cobwebs were also collected from 3 of the stalls. One of these

specimens was positive. It was from a stall which housed 2 horses (K-1B and K-2B). Neither of these horses had R. equi in their feces.

Selective Media

Colony Characteristics

The recovery of one or more colonies of R. equi on a single agar plate constituted a positive sample. Colonies were black or gray on all of the selective mediums.

During the first week of incubation, colonies on Dextrose Proteose agar were 5-13 mm in diameter, wet, mucoid and brownish black. At that same time interval, colonies on NANAT agar were 2-5 mm in diameter, smooth, rounded and light gray with a white periphery or 4-7 mm in diameter, wet, dark gray and had a teardrop appearance. Likewise, colonies on M3T were 3-9 mm in diameter, blackish-gray, transparent and wet or had a teardrop appearance.

After 3 weeks of incubation, most colonies on all mediums were flat, dull black and dry in appearance but still had a butyrous consistency when picked with a loop. The colonies which were gray and rounded with a white periphery on NANAT did not change their appearance to any great extent during this time interval. Some colonies which were viable on M3T at 1 week incubation had died after 3 weeks.

Recovery of *R. equi* from Selective MediumsFecal Specimens

Data on the effectiveness of the various isolation schemes for recovery of *R. equi* from equine fecal material are presented in Tables 7 and 8. No single selective isolation scheme was uniformly successful. However, a combination of the methods DEX, NA, M3T, B64, TNA4 and TNA7 detected all of the isolates.

The most successful method was direct plating of feces onto M3T medium. Thirty isolates (65.2 % of all isolates) were recovered by this method. On sixteen (34.8 %) occasions, it was the only medium on which isolation was accomplished.

Feces enrichment in BHI-6 broth with subculture to Dextrose Proteose Agar after 4 days incubation detected 4 additional isolates. In one instance, this was the only method which was successful. Use of TAMP broth with subculture to NANAT Agar after 4 days incubation detected 5 additional isolates not detected by the above methods. In 3 instances, this was the only medium on which the organism was isolated.

Enrichment of feces in TAMP broth with subculture to NANAT after 7 days incubation detected 3 additional isolates. This was the only method by which 2 positive samples were detected. Direct plating of feces onto DEX and NANAT each detected 1 isolate not recovered by above

Table 7. Frequency of Isolation of *Rhodococcus equi* from Feces with Selective Schemes Utilized.

Isolation Scheme	Total Isolations	This System only
1. Direct plating of feces onto Oex	4	1
2. Direct plating of feces onto NANAT agar.	1	1
3. Direct plating of feces onto M3T agar.	30	16
4. Feces enrichment in BHI-6 broth subculture to Dex after 4 days incubation.	6	1
5. Feces enrichment in BHI-6 broth subculture to Oex after 7 days incubation.	0	0
6. Feces enrichment in BHI-12+2 broth subculture to Oex after 4 days incubation.	5	0
7. Feces enrichment in BHI-12+2 broth subculture to Oex after 7 days incubation.	0	0
8. Feces enrichment in TAMP broth, subculture to NANAT after 4 days incubation.	9	3
9. Feces enrichment in TAMP broth, subculture to NANAT after 7 days incubation.	8	2
10. Feces enrichment in TAMP broth, subculture to M3T after 4 days incubation.	8	0
11. Feces enrichment in TAMP broth, subculture to M3T after 7 days incubation.	3	0

- M3T = M3 MEDIUM OF ROWBOTHAM AND CROSS, 1977 PLUS POTASSIUM TELLURITE (BARTON AND HUGHES, 1981)
- NANAT = NALIDIXIC ACID, NOVOBIOICIN, CYCLOHEXIMIDE, POTASSIUM TELLURITE MEDIUM (WOOLCOCK ET AL., 1979)
- OEX = DEXTROSE PROTEOSE # 3 AGAR, POLYMYXIN-B, AMPHOTERICIN-B, POTASSIUM TELLURITE, STERILE CHOCOLATIZED BOVINE BLOOD (SMITH AND ROBINSON, 1981)
- TAMP = TRYPTICASE SOY BROTH, CYCLOHEXIMIDE, NALIDIXIC ACID, PENICILLIN (BARTON AND HUGHES, 1981)
- BHI-6 = BRAIN HEART INFUSION BROTH, POLYMYXIN-B (SMITH AND ROBINSON, 1981)
- BHI-12+2 = BRAIN HEART INFUSION BROTH, POLYMYXIN-B, AMPHOTERICIN-B (SMITH AND ROBINSON, 1981)

Table 8. Selective Isolation Schemes Which Were Successful in Recovery of Rhodococcus equi from Equine Feces.

Horse Identification Number	Isolation Scheme											
	DEX	NA	M3T	B64	B67	BL24	BL27	TNA4	TNA7	TM4	TM7	
H-7	+	-	-	-	-	-	-	-	-	-	-	
H-9	-	-	+	-	-	-	-	-	-	-	-	
H-12	-	-	-	-	-	-	-	-	+	-	-	
H-13	-	-	+	-	-	-	-	-	-	-	-	
H-17	-	-	+	-	-	-	-	-	+	-	-	
H-20	-	-	+	-	-	-	-	-	-	-	-	
H-24	-	-	+	-	-	-	-	-	-	-	-	
H-26	-	-	+	-	-	-	-	-	-	-	-	
C-1	-	-	+	-	-	-	-	-	+	-	-	
C-3	-	-	+	-	-	-	-	-	+	-	-	
C-7	-	-	+	-	-	-	-	-	-	-	-	
C-9	-	-	+	-	-	-	-	+	-	+	-	
C-11	-	-	+	-	-	-	-	-	-	-	-	
C-12	-	-	+	-	-	-	-	-	-	-	-	
C-13	-	-	+	-	-	-	-	-	-	-	-	
C-14	-	-	+	-	-	-	-	-	-	-	-	
C-15	-	-	+	-	-	-	-	-	+	+	-	
C-16	-	-	+	-	-	-	-	-	-	-	-	
C-17	-	-	+	-	-	-	-	+	+	-	-	
C-18	-	-	+	-	-	-	-	+	+	-	-	
C-20	-	-	+	-	-	-	-	+	+	-	-	
C-22	-	-	+	-	-	-	-	-	-	-	-	
C-23	-	-	-	-	-	-	-	+	-	-	-	
K-0A	-	-	-	+	-	+	-	+	-	-	-	
K-4B	-	-	-	-	-	-	-	+	-	-	+	

Table 8 (continued) KEY TO ABBREVIATIONS:

- OEX = OEXTROSE PROTEOSE # 3 AGAR, POLYMYXIN-B, AMPHOTERICIN-B, POTASSIUM TELLURITE, STERILE CHOCOLATIZED BOVINE BLOOD (SMITH AND ROBINSON, 1981) Specimen plated directly on this medium.
- NA = (NANAT) NALIDIXIC ACID, NOVIOBIACIN, CYCLOHEXIMIDE, POTASSIUM TELLURITE MEDIUM (WOOLCOCK ET AL., 1979) Specimen plated directly on this medium.
- M3T = M3 MEDIUM OF ROWBOTHAM AND CROSS, 1977 PLUS POTASSIUM TELLURITE (BARTON AND HUGHES, 1981) Specimen plated directly on this medium.
- B64 = (BHI-6) BRAIN HEART INFUSION BROTH, POLYMYXIN-B (SMITH AND ROBINSON, 1981) Specimen incubated in this broth for 4 days and then broth plated onto DEX agar.
- B67 = (BHI-6) BRAIN HEART INFUSION BROTH, POLYMYXIN-B (SMITH AND ROBINSON, 1981) Specimen incubated in this broth for 7 days and then broth plated onto DEX agar.
- B124 = (BHI-12+2) BRAIN HEART INFUSION BROTH, POLYMYXIN-B, AMPHOTERICIN-B (SMITH AND ROBINSON, 1981) Specimen incubated in this broth for 4 days and then broth plated onto DEX agar.
- B127 = (BHI-12+2) BRAIN HEART INFUSION BROTH, POLYMYXIN-B, AMPHOTERICIN-B (SMITH AND ROBINSON, 1981) Specimen incubated in this broth for 7 days and then broth plated onto DEX agar.
- TNA4 = (TANP) TRYPTICASE SOY BROTH, CYCLOHEXIMIDE, NALIDIXIC ACID, PENICILLIN (BARTON AND HUGHES, 1981) Specimen incubated in this broth for 4 days and then broth plated onto NANAT agar.
- TNA7 = (TANP) TRYPTICASE SOY BROTH, CYCLOHEXIMIDE, NALIDIXIC ACID, PENICILLIN (BARTON AND HUGHES, 1981) Specimen incubated in this broth for 7 days and then broth plated onto NANAT agar.
- TM4 = (TANP) TRYPTICASE SOY BROTH, CYCLOHEXIMIDE, NALIDIXIC ACID, PENICILLIN (BARTON AND HUGHES, 1981) Specimen incubated in this broth for 4 days and then broth plated onto M3T agar.
- TM7 = (TANP) TRYPTICASE SOY BROTH, CYCLOHEXIMIDE, NALIDIXIC ACID, PENICILLIN (BARTON AND HUGHES, 1981) Specimen incubated in this broth for 7 days and then broth plated onto M3T agar.

methods.

Environmental Samples

Data on the effectiveness of the various isolation schemes for recovery of R. equi from environmental samples are presented in Tables 9 and 10. No single selective isolation scheme was uniformly successful. When isolations were successful, they were by only one of the schemes.

A total of 9 isolations were made on 6 different mediums. The medium which was most successful was M3T on which 3 of the recoveries were made. The only other method to detect more than one isolate was TM7. A combination of the methods M3T, B67, B124, TNA4, TNA7 and TM7 detected all of the isolates. The methods B67, B124 or TM7 were not useful in detection of R. equi from feces but were successful in isolation of the organism from soil and cobwebs. No isolations were made from air circulation filters in the stall areas of the KSU Veterinary Hospital.

Identification of Isolates

Results of biochemical tests utilized to identify the isolates as R. equi are presented in Table 11. Of the selected tests utilized in identification, results generally agreed with previously reported reactions (Weaver et al., 1974). The only discrepancies were that two of the isolates were esculin positive and 6 of the strains were nitrate negative.

Table 9. Frequency of Isolation of *Rhodococcus equi* From Environmental Samples With Selective Schemes.

Isolation Scheme	Total Isolations	This System only
1. Direct plating of sample onto Dex	0	0
2. Direct plating of sample onto NANAT agar.	0	0
3. Direct plating of sample onto M3T agar.	3	3
4. Enrichment in BHI-6 broth subculture to Oex after 4 days incubation.	1	1
5. Enrichment in BHI-6 broth subculture to Oex after 7 days incubation.	1	1
6. Enrichment in BHI-12+2 broth subculture to Dex after 4 days incubation.	1	1
7. Enrichment in BHI-12+2 broth subculture to Oex after 7 days incubation.	0	0
8. Enrichment in TANP broth, subculture to NANAT after 4 days incubation.	1	1
9. Enrichment in TANP broth, subculture to NANAT after 7 days incubation.	1	1
10. Enrichment in TANP broth, subculture to M3T after 4 days incubation.	0	0
11. Enrichment in TANP broth, subculture to M3T after 7 days incubation.	2	2

- M3T = M3 MEDIUM OF ROWBOTHAM AND CROSS (1977) PLUS POTASSIUM TELLURITE (BARTON AND HUGHES, 1981)
- NANAT = NALIDIXIC ACID, NOVIOBIOCIN, CYCLOHEXIMIDE, POTASSIUM TELLURITE MEDIUM (WOOLCOCK ET AL., 1979)
- DEX = DEXTROSE PROTEOSE # 3 AGAR, POLYMYXIN-B, AMPHOTERICIN-B, POTASSIUM TELLURITE, STERILE CHOCOLATIZED BOVINE BLOOD (SMITH AND ROBINSON, 1981)
- TANP = TRYPTICASE SOY BROTH, CYCLOHEXIMIDE, NALIDIXIC ACID PENICILLIN (BARTON AND HUGHES, 1981)
- BHI-6 = BRAIN HEART INFUSION BROTH, POLYMYXIN-B (SMITH AND ROBINSON, 1981)
- BHI-12+2 = BRAIN HEART INFUSION BROTH, POLYMYXIN-B, AMPHOTERICIN-B (SMITH AND ROBINSON, 1981)

Table 10. Key to Abbreviations:

DEX	= DEXTROSE PROTEOSE # 3 AGAR, POLYMYXIN-B, AMPHOTERICIN-B, POTASSIUM TELLURITE, STERILE CHOCOLATIZED BOVINE BLOOD (SMITH AND ROBINSON, 1981) Specimen plated directly on this medium.
NA	= (NANAT) NALIDIXIC ACID, NOVOBIOCIN, CYCLOHEXIMIDE, POTASSIUM TELLURITE MEDIUM (WOOLCOCK ET AL., 1979) Specimen plated directly onto this medium.
M3T	= M3 MEDIUM OF ROWBOTHAM AND CROSS (1977) PLUS POTASSIUM TELLURITE (BARTON AND HUGHES, 1981) Specimen plated directly onto this medium.
B64	= (BHI-6) BRAIN HEART INFUSION BROTH, POLYMYXIN-B (SMITH AND ROBINSON, 1981) Specimen incubated in this broth for 4 days and then broth plated onto DEX agar.
B67	= (BHI-6) BRAIN HEART INFUSION BROTH, POLYMYXIN-B (SMITH AND ROBINSON, 1981) Specimen incubated in this broth for 7 days and then broth plated onto DEX agar.
B124	= (BHI-12+2) BRAIN HEART INFUSION BROTH, POLYMYXIN-B, AMPHOTERICIN-B (SMITH AND ROBINSON, 1981) Specimen incubated in this broth for 4 days and then broth plated onto DEX agar.
B127	= (BHI-12+2) BRAIN HEART INFUSION BROTH, POLYMYXIN-B, AMPHOTERICIN-B (SMITH AND ROBINSON, 1981) Specimen incubated in this broth for 7 days and then broth plated onto DEX agar.
TNA4	= (TANP) TRYPTICASE SOY BROTH, CYCLOHEXIMIDE, NALIDIXIC ACID, PENICILLIN (BARTON AND HUGHES, 1981) Specimen incubated in this broth for 4 days and then broth plated onto NANAT agar.
TNA7	= (TANP) TRYPTICASE SOY BROTH, CYCLOHEXIMIDE, NALIDIXIC ACID, PENICILLIN (BARTON AND HUGHES, 1981) Specimen incubated in this broth for 7 days and then broth plated onto NANAT agar.
TM4	= (TANP) TRYPTICASE SOY BROTH, CYCLOHEXIMIDE, NALIDIXIC ACID, PENICILLIN (BARTON AND HUGHES, 1981) Specimen incubated in this broth for 4 days and then broth plated onto M3T agar.
TM7	= (TANP) TRYPTICASE SOY BROTH, CYCLOHEXIMIDE, NALIDIXIC ACID, PENICILLIN (BARTON AND HUGHES, 1981) Specimen incubated in this broth for 7 days and then broth plated onto M3T agar.

Table 10. Selective Isolation Schemes Which Were Successful in Recovery of Rhodococcus equi from Environmental Samples.

Sample Number	Type of Sample	Isolation Scheme									
		DEX	NA	M3T	B64	B67	BI24	BI27	TNA4	TNA7	TM4 TM7
H-S-3	(cobwebs)	-	-	+	-	-	-	-	-	-	-
H-S-5	(soil)	-	-	+	-	-	-	-	-	-	-
C-S-16	(soil)	-	-	-	-	-	-	-	-	-	+
C-S-19	(soil)	-	-	-	-	-	-	-	+	-	-
K-S-1	(cobwebs)	-	-	+	-	-	-	-	-	-	-
K-S-5	(soil)	-	-	-	-	+	-	-	-	-	-
K-S-10	(soil)	-	-	-	-	-	-	-	-	+	-
K-S-11	(soil)	-	-	-	-	-	+	-	-	-	-
D-S-20	(soil)	-	-	-	-	-	-	-	-	-	+
TOTAL POSITIVE		0	0	3	0	1	1	0	1	1	0 2
ON THIS MEDIUM ONLY		0	0	3	0	1	1	0	1	1	0 2

See: Table 5 for details of media composition.

Table 11. Results of Biochemical Reactions of Rhodococcus equi Strains Isolated from Feces or Environmental Samples.

Biochemical Test	Expected Result	Number Positive	Number Negative
Gram Stain	Gram positive	55	0
Catalase	Positive	55	0
O-F Glucose	Alkaline	55	0
Heart Infusion Slant	Moist to runny pinkish growth	55	0
CAMP	Positive	55	0
Gelatin Hydrolysis	Negative	55	0
Casein Hydrolysis	Negative	55	0
Urea	Variable	55	0
Esculin Hydrolysis	Negative	54	1
Nitrate	Variable	49	6

Serotyping of IsolatesAgar Gel Double Immunodiffusion

None of the antisera which were collected following the first inoculation of the rabbits formed homologous precipitin bands in immunodiffusion tests when tested with antigens prepared by the methods of Prescott (1981a). Antisera against the 7 serotypes which were donated by Prescott^a formed distinct homologous bands with antigens of serotypes 1, 2, 3 and 4 but not with antigens of serotypes 5, 6 and 7. Antisera which was collected 5-7 days following the second inoculation also produced no precipitin bands with antigen prepared by the same method. However, using antigen prepared by the alternative method, weak homologous precipitin bands were observed with anti-serotype 2.

Antisera against all 7 serotypes collected following the third inoculation formed weak homologous precipitin bands which were difficult to read. At this point, rabbits were administered an additional inoculation of the antigen in an attempt to increase the clarity of the test. Antisera collected after this inoculation formed homologous precipitin bands and heterologous bands which were indistinguishable from homologous. Since results of serotyping utilizing gel diffusion were inconclusive and consumed much time, counterimmunoelectrophoreses (CIEP) was adopted as the method to be used for further serotyping.

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Serotyping by Counterimmunoelectrophoresis

Unadsorbed antisera of each serotype formed heterologous precipitin bands with all preparations of antigens of each of the 7 serotypes. Dilution of antigens and/or antisera diminished the intensity of cross reactions, but they were not eliminated. Fifty percent saturation of the antisera with ammonium sulfate and reconstitution of the precipitate to the original volume did not affect the number of homologous or heterologous precipitin bands formed.

In attempts to remove the heterologous precipitin reactions, antisera was adsorbed with a variety of different serotypes and by two different methods. Details of adsorption studies which were conducted on the antisera are presented in Tables 12, 13, 14, 15, 16, 17 and 18. Adsorption of antisera with cells of serotype 1 proved difficult. The cells of this strain were heavily encapsulated and the capsular material eluted from the cells during incubation of the cells with the serum. This resulted in an extremely viscous preparation which precluded use in CIEP tests after two adsorptions. Multiple adsorptions with either whole cells or antigen adsorbed to DEAE of other serotypes in all but one instance failed to remove heterologous bands and retain homologous bands. Anti-serotype 1 when adsorbed two times with serotype 2 cells, four times with serotype 4 cells and two times with serotype 7 cells formed weak precipitin bands with only

Table 12. Counterimmunoelectrophoresis Serotyping Results Obtained with Antisera to *Rhodococcus equi* Serotype 1 Adsorbed with Cells of Serotypes 2, 4, and 7.

Adsorbing Serotype/s	Number of Adsorptions	Reactions With Serotypes		
		Strong	Weak	Faint
2	2	1,4,5,6,7	3	-
2	2			2
4	2	1,4	7	-
				2,3,5,6
2	2			
4	4	1,4	7	-
				2,3,5,6
2	2			
4	4			
7	2	-	1	-
				2,3,4,5,6,7
2 ^a	2			
7	2	1,4	-	5,2
				3,6,7
2 ^b	1	1,2,3,4,6,7	5	-
				-
2 ^c	1	-	1,2,3,4,7	5
				6

a = 1:2 and 1:4 dilutions of antiserum were electrophoresed against the highest dilution of each antigen that caused a homologous reaction.

b = Serum adsorbed with DEAE previously saturated with antigen 2.

c = Serum precipitated with ammonium sulfate and adsorbed with DEAE previously saturated with antigen 2.

Table 13. Counterimmunoelectrophoresis Serotyping Results Obtained with Antisera to *Rhodococcus equi* Serotype 2 Adsorbed with Cells of Serotypes 1, 4, and 7.

Adsorbing Serotype	Number of Adsorptions	Reactions With Serotypes			
		Strong	Weak	Faint	None
1	1	1, 2	3, 7	5, 6	4
1	2	1, 2, 4	5	3, 6, 7	-
1	2				
4	2	-	1, 2, 4	-	3, 5, 6, 7
a 4	2				
7	2	1, 4	-	2	3, 5, 6, 7
b 1	1	-	1, 2, 7	-	3, 4, 5, 6

a = 1:2 and 1:4 dilutions of antiserum were electrophoresed against the highest dilution of each antigen that caused a homologous reaction.

b = Serum precipitated with ammonium sulfate and adsorbed with DEAE previously saturated with antigen 1.

Table 14. Counterimmunoelectrophoresis Serotyping Results Obtained with antisera to *Rhodococcus equi* Serotype 3 Adsorbed with Cells of Serotypes 1, 2, 4, and 7.

Adsorbing Serotype/s	Number of Adsorptions	Reactions with Serotypes			
		Strong	Weak	Faint	None
1	1	1,3,4,7	-	-	2,5,6
1	2	1,3,7	-	-	2,4;5,6
1	2				
4	2	1,3,4,7	-	6	2,5
2	2				
4	2				
7	2	-	-	-	1,2,3,4,5,6,7
a	2				
7	2	1,3,4	2	5	6,7
b	1	-	2,3,4,6,7	-	1,5

a = 1:2 and 1:4 dilution of antiserum were electrophoresed against the highest dilution of each antigen that caused a homologous reaction.
b = Serum precipitated with ammonium sulfate and adsorbed with DEAE previously saturated with antigen 1.

Table 15. Counterimmunoelectrophoresis Serotyping Results Obtained with Antisera to *Rhodococcus equi* Serotype 4 Adsorbed with Cells of Serotypes 1, 2, and 7.

Adsorbing Serotype/s	Number of Adsorptions	Reactions With Serotypes			
		Strong	Weak	Faint	None
1	1	1,4,7	-	2,3	5,6
1	2	1,4	3,5,6,7	-	2
a 2	2	1,4,5	-	2,7	3,6
7	2				
b 1	1	1,3,6	2,4,5,7	-	-

a = 1:2 and 1:4 dilution of antiserum were electrophoresed against the highest dilution of each antigen that caused a homologous reaction.

b = Serum precipitated with ammonium sulfate and adsorbed with DEAE previously saturated with antigen 1.

Table 16. Counterimmunoelectrophoresis Serotyping Results Obtained with Antisera to *Rhodococcus* equi Serotype 5 Adsorbed with Cells of Serotypes 1, 2, 4, and 7.

Adsorbing Serotype/s	Number of Adsorptions	Reactions With Serotypes			
		Strong	Weak	Faint	None
1	1	1,4	-	3,5,6,7	2
2	2	1,4	7	-	2,3,5,6
2	2				
4	2	-	1,4	5	2,3,6,7
a 2	2				
7	2	1,4,5	-	-	2,3,6,7
b 1	1	-	2,3,4,6,7	-	1,5

a = 1:2 and 1:4 dilutions of antiserum were electrophoresed against the highest dilution of each antigen that caused a homologous reaction.

b = Serum precipitated with ammonium sulfate and adsorbed with DEAE previously saturated with antigen 1.

Table 17. Counterimmunoelectrophoresis Serotyping Results Obtained with Antisera to *Rhodococcus equi* Serotype 6 Adsorbed with Cells of Serotypes 1, 2, 4, and 7.

Adsorbing Serotype/s	Number of Adsorptions	Reactions With Serotypes			
		Strong	Weak	Faint	None
1	1	1,4,7	6	2,3,5	-
2	2	1,4,5,6,7	3	-	2
2	2				
4	2	1,4,7	3,6	-	2,5
a	2				
7	2	1,4	2,5	7	3,6
b	1	1,2,3,4,6,7	5	-	-

a = 1:2 and 1:4 dilutions of antiserum were electrophoresed against the highest dilution of each antigen that caused a homologous reaction.

b = Serum precipitated with ammonium sulfate and adsorbed with DEAE previously saturated with antigen 1.

Table 18. Counterimmunoelectrophoresis Serotyping Results Obtained with Antisera to Rhodococcus equi Serotype 7 Adsorbed with Cells of Serotypes 1, 2, and 4.

Adsorbing Serotype/s	Number of Adsorptions	Reactions With Serotypes			
		Strong	Weak	Faint	None
1	1	1,4,7	-	2,3,5,6	-
2	2	1,4	3,7	5	2,6
2 4	2 1	1,4	7	3	2,5,6
2 4	2 3	1,4	7	-	2,3,5,6
a 2 4	2 2	1,4,5	7	-	2,3,6
b 1	1	1,4,5,6,7	2,3	-	-

a = 1:2 and 1:4 dilutions of antiserum were electrophoresed against the highest dilution of each antigen that caused a homologous reaction.

b = Serum precipitated with ammonium sulfate and adsorbed with DEAE previously saturated with antigen 1.

serotype 1 antigen (Table 12). Due to the extreme difficulties encountered in attempting to produce only homogeneously reacting antisera, none of the R. equi fecal or environmental isolates were examined to determine their serotype.

DISCUSSION

An objective of this project was to compare the incidence of R. equi on farms with and without a history of foal pneumonia caused by this organism. The owners of the farms requested anonymity when this study was initiated. Consequently, names of the owners of the farms are not included in this thesis.

Woolcock et al. (1979) isolated R. equi from 98 of 127 (78.9 %) horse fecal samples examined. In this study, the incidence of R. equi in horse feces from all horses examined was not as high (48.4 %). However, Woolcock et al. (1979) did not differentiate between incidence in hospitalized and non-hospitalized horses. Horses which were at the KSU Veterinary Hospital had a higher (88.9 %) incidence of R. equi and horses which were on farms had a lower (31.2 %) incidence. There was a significantly higher ($p = 0.000$) proportion of culture positive R. equi horses that were sampled in the KSU Veterinary Hospital than those sampled on farms.

Barton and Hughes (1981a) suggested that their results indicated a lower incidence of R. equi in feces than Woolcock et al. (1979) because the latter study failed to differentiate between samples from dung pads and rectally collected samples. Colony counts of R. equi per gram of feces increased 10,000 fold by three weeks after collection and remained high for at least 2 more weeks (Barton and

Hughes 1981b). In this study, defecated samples were less than 6 hours old to avoid the dramatic increase in organism numbers. No effort was made to record rectally collected vs. defecated samples either on farms or in the hospital. However, a higher number of samples collected from Hospitalized Horses were defecated while a higher number of rectally collected samples were from farms. This may be the reason for the significantly higher proportion of isolations from hospitalized horses than from non-hospitalized horses. If true, this would support the findings of Barton and Hughes (1981b) that R. equi numbers increase in dung pads.

An interesting future study would be to study numbers of R. equi in fecal samples collected rectally in comparison to voided samples from the same horse which were allowed to incubate at room temperature for 2, 4 and 6 hours. Because of the ability of R. equi to increase in numbers in dung pads (Barton and Hughes, 1981b), a higher incidence of R. equi in all horses may have been detected in this study if fecal samples were allowed to incubate.

Barton and Hughes (1981b) recovered R. equi from rectally collected feces of grazing horses, but not from penned horses. When results from all non-nursing horses in this study were analyzed statistically, the type of feed consumed did not influence the recovery rate. On the Previously Infected Farm, 14 of 17 (82.3 %) non-nursing horses with fecals positive for R. equi were on pasture

while only 1 of the 7 (14.3 %) penned horses was positive which would tend to agree with their results. However, horses which were sampled at the KSU Veterinary Hospital were all penned and 17 of 21 (80.9 %) of these horses were culture positive. As mentioned previously, culture results from the hospitalized horses may have been influenced by the fact that a majority of those specimens were voided feces. One might argue that duration of stay in the hospital might influence the culture result, but positive horses had been patients from 1 to 83 days. Results obtained do not support the premise that ration consumed influences the rate of recovery of R. equi from equine feces.

It was anticipated that the recovery rate of R. equi on the Control Farm would be lower than that on the infected farms and the Previously Infected Farm. This did not prove to be the case. The rate of recovery on the Control Farm was significantly less ($p = 0.025$) than on the Previously Infected Farm. However, it was greater (38.8 %) but not significantly different ($p = 0.155$) from the two infected farms (11.8 % and 22.2 %). The rate of recovery on the Previously Infected Farm was significantly greater than on any of the other farms. The reason/s for these results are not clear.

The Control Farm was chosen to survey the incidence and serotypes of R. equi on a farm which had experienced no previous R. equi problems. Any foal pneumonias which had

occurred in the last few years had been diagnosed by the KSU Veterinary Hospital as due to problems other than R. equi. The owner shipped a few mares away from the farm for breeding, but no "outside" mares were brought in from other farms to be bred. The horses were healthy and were provided with high quality hay or pasture, plus grain. However, the Control Farm ranked next after the Previously Infected Farm in the percentage of R. equi culture positive fecal specimens.

Because of the similarity in isolation rates between the Control Farm and the infected farms, other conditions may play a role in onset of foal pneumonia. Maintenance of optimal health with an adequate immunization and parasite control program to eliminate predisposing factors may be one of these conditions. Bain (1963) observed that R. equi foal pneumonia disappeared from studs in Australia where the disease had previously been endemic when an adequate parasite control program was initiated. Knight (1969) stated that foals which contracted R. equi pneumonia often had a preceding viral respiratory infection or were weak foals at birth.

The parasite control and vaccination programs on the Control Farm appeared adequate. Infected Farm I had no regular parasite control program until after the R. equi outbreak. Elimination of predisposing factors by initiation of a parasite control and vaccination program appeared to

reduce the incidence of R. equi infections. One year after the samples were collected, a foal which had combined immunodeficiency (CID) died of an R. equi infection. This was the only foal which was affected that year. This farm was originally selected to be sampled when a foal which was an offspring of the same mare was suspected to have an R. equi infection. This foal (K-0-A), subsequently was found to have R. equi in its feces and died of a confirmed R. equi infection. The immune status of this foal was not determined at that time nor could it be determined subsequently because serum was not available. That this foal may have had CID is possible since defects in the immune system may predispose to R. equi infections. The dam of these foals was fecal culture negative for R. equi on 2 separate occasions.

Infected Farm II appeared to have an adequate parasite control and immunization program. The single foal that succumbed to an R. equi infection on this farm had been shipped to two breeding farms in Texas with the dam when less than 1 week old. Three of the 4 remaining foals at Infected Farm II had mild respiratory problems at the time of sampling. One year later, none of these foals had died.

The Previously Infected Farm, which experienced an outbreak of R. equi infections three years before this sampling, had no specific immunization and parasite control program prior to the outbreak. During and after the

outbreak, their veterinarian initiated a vaccination and parasite control program to decrease predisposing disease factors in the herd. Since that time, the farm had experienced no losses due to R. equi infections.

Hutchins et al. (1988) reported the breeds of 40 R. equi infected foals. Sixty-five percent were Thoroughbreds, 27.5 % Arabians and 7.5 % Standardbreds. Wilks et al. (1982) suggested that these figures reflected the proportion of breeds in the horse population, not a breed susceptibility. The results of this study tended to agree with the conclusions of Wilks et al. (1982). Statistical evaluation of isolation rates from various breeds appeared to indicate only a farm effect, because only one breed predominated on most farms. A sufficient number of Thoroughbreds and Quarter Horses were present on the Control Farm to conduct a statistical analysis. There was no significant difference between the breeds ($p = 0.761$).

There was no apparent significant effect of sex of the animal sampled upon the isolation rate. However, horses which contracted an R. equi infection as a foal, dams of these foals or half-siblings of these foals had significantly higher isolation rates ($p = 0.021$). Since many horses on the Previously Infected Farm were related and were culture positive, this relationship may have skewed this statistical evaluation. An interesting future study might be to examine fecal culture rates and the incidence of

infection in particular lines of horses. If this trend could be substantiated, certain susceptible lines of animals might be detected and eliminated or special care provided to foals.

Robinson (1982) cultured soil from 6 different farms where infections from R. equi were and were not endemic. His results indicated that R. equi was normally present in soil on farms where R. equi infections were endemic, but not on farms where it was not endemic. Of all environmental samples (soil and cobwebs) he cultured, he had a success rate of 32.9 %. Others (Wilson, 1955; Bain, 1963; and Knight, 1969) have also indicated that soil was an important reservoir. In this study, a relatively low isolation rate (14.9 %) from soil was obtained. The Control Farm and the Previously Infected Farm which had the highest isolation rates from fecal material both had an isolation rate of 16.6 %. On these farms where horses were shedding the organism in their feces at a reasonably high rate the organism apparently did not survive in the soil for an extended period of time. This may possibly be due to the soil type. Barton and Hughes (1981b) indicated that R. equi was isolated at a lower rate from neutral or alkaline and clay-based soils. Soils in this region of Kansas tend to be alkaline in nature due to extensive limestone deposits. However, Robinson (1982) also indicated that his soil samples consisted of "top soil, often mixed with dried

faeces and bedding material". Considering that Barton and Hughes (1981b) indicated that numbers of R. equi increased dramatically in voided fecal material, some of Robinson's soil isolates may well have been from fecal material and not from soil.

Smith and Robinson (1981) and Robinson (1982) also cultured cobwebs from box stalls for the presence of R. equi. It is difficult to interpret the actual number of isolates they obtained from this source by reading the articles, but they indicated that the organism was cultured. Only 2 of 33 samples of cobwebs yielded cultures of R. equi in this study. Of 13 cobweb samples cultured from the Previously Infected Farm, no cultures were recovered. Yet this farm had 15 of 24 horses with R. equi in their feces. From these results, it would appear that cobwebs are not an important source for infection of foals.

A surprising result was that the KSU Veterinary Hospital was the only location at which no R. equi was isolated from the environment, since this is the location at which the highest rate of recovery from feces was made. Possible reasons why all dust samples collected from filters were negative for R. equi are: (1) Since the dust samples were not collected at the same time that fecal samples were collected, R. equi was possibly not being shed by any of the horses when the dust was collected. Based on studies done by other workers (Woolcock et al., 1980 and Barton and

Hughes, 1981a), this is not a likely possibility. (2) Rhodococcus equi was not aerosolized and drawn up into the filters. Since R. equi can be isolated from cobwebs containing contaminated dust (Smith and Robinson, 1981), then the organism should have been carried by dust onto the filters. (3) The mesh size of the filters was not sufficiently small to retain the organism. However, an abundance of dust particles was retained on the filters and the likelihood that all organisms could have passed through the filters in the airstream is remote. (4) Media compositions were incorrect for isolation of the organism. Media used to plate the dust were also utilized for other samples which yielded isolates. Therefore, a media failure is not likely. (5) The most likely possibility is that viable R. equi were not present in dust carried to the filters. Non-viability might possibly have been due to disinfectants which were used in the stall areas.

The possibility that both wild and domestic fowl may serve as a reservoir of infection for R. equi should be investigated further. Colonization of the avian intestinal tract by R. equi has not been documented. Barton and Hughes (1981a) examined two chicken fecal specimens, but individual results were not reported. Infected Farm I had many chickens, ducks and wild sparrows living among the horses in the barn and paddocks. Cloacal samples collected from a baby sparrow and a chicken in the barn yielded cultures of

R. equi. These birds may have ingested the organism by eating feed from horse feces or from soil as they foraged for seeds or insects. Recovery of R. equi from these birds may represent colonization or only survival of the organism as it passed through the intestinal tract.

Because of the diverse population of bacteria present in fecal material and environmental samples cultured for R. equi, investigators have examined various selective mediums and isolation schemes to enhance recovery of pure cultures of the organism (Rowbotham and Cross, 1977; Woolcock et al., 1979; Barton and Hughes, 1981a; and Smith and Robinson, 1981). Selective mediums and isolation schemes proven useful to others were chosen for use in this study. Minor modifications of mediums and schemes were done when it appeared that the previous investigators did not have great success or their results were not easily repeated.

Rowbotham and Cross, (1977) developed a selective agar medium for isolation of Rhodococcus coprophilus from ruminant feces which they named M3. This medium was complex and contained KH_2PO_4 , Na_2HPO_4 , KNO_3 , NaCl , MgSO_4 , CaCO_3 , FeSO_4 , ZnSO_4 , MnSO_4 , Sodium Propionate, Thiamin and Cycloheximide. This medium was modified by addition of potassium tellurite for isolation of R. equi by Barton and Hughes (1981a) and renamed M3T. Tellurite caused bacteria, including R. equi, which reduced the compound to appear black. They utilized this medium in attempts to recover the

organism from feces of a variety of species of animal and soil. It is difficult to interpret their results in regard to the efficiency of this medium in comparison to other mediums. However, they stated that "A combination of TANP broth culture and direct plating onto M3T medium seems to be the best approach to examining rectal feces or large bowel contents for R. equi".

Results of this investigation indicated that direct plating of feces onto this medium was the single most effective method for recovery of R. equi. Thirty (26.3 %) isolations were made from the 114 fecal samples collected. Of all isolations of R. equi (46) from feces 65.2 % were made on this medium and in 16 instances, this was the only medium on which isolation was achieved. A disadvantage to use of this medium is that preparation is tedious and time consuming. However, from these results, it would appear that the benefits outweigh the difficulties.

Only 18 of the 46 (21.7 %) fecal isolates were recovered after TANP broth was incubated 4 and 7 days and then subcultured to M3T, even though M3T was the most useful medium for direct plating of fecal samples. This apparent discrepancy may have been due to the paucity of inhibitory substances in M3T, allowing overgrowth of R. equi colonies by other bacteria which had also multiplied in the TANP enrichment broth. This finding may disagree with results obtained by Barton and Hughes (1981a), because they stated

that a combination of direct plating of M3T and their TANP scheme was most useful for recovery of R. equi from feces. However, their TANP scheme included Tinsdale medium and their fecal samples were primarily from ruminants.

In addition to the above described scheme, a TANP scheme not previously described in the literature in which TANP broth was subcultured to NANAT after 4 and 7 days incubation (TNA4 and TNA7), was successful in recovery of 16 of the 46 fecal isolates obtained. Possibly, since NANAT contained more inhibitory compounds than M3T, growth of fewer contaminants allowed colonies of R. equi to be recognized more easily. This scheme detected 5 isolates not detected by the TANP to M3T (TM4 and TM7) scheme or by direct plating of M3T.

Few soil and cobweb isolates were obtained, so evaluation of selective media is difficult. Direct plating of M3T again appeared to be the single most effective medium for isolation of R. equi, resulting in recovery of 3 of the 9 environmental isolates. TANP enrichment (TNA4, TNA7, TM4, TM7) enabled detection of 4 more isolates. Barton and Hughes (1981a) reported that culturing in TANP broth was superior to direct plating for isolation of R. equi from soil samples, which the results of this study did not support or refute.

The media least useful in this investigation were those employing polymyxin B and amphotericin B as inhibitors (Dex,

B64, B67, B124, B127). Only 2 fecal and 2 soil isolates were obtained exclusively on media in this scheme. Smith and Robinson (1981) decontaminated samples by physical or chemical processes before inoculation of selective media containing the same inhibitors. Broths and agars in this study were inoculated directly with specimens. This difference in specimen treatment may be one reason that overgrowth by contaminants was such a problem with these media.

Another reason why the number of R. equi isolates recovered upon subculture after incubation in selective broth may have been artificially low was because of the tendency for sample material to rise to the top of the broth and seal the tube. This closed environment in sealed tubes may have created an atmosphere lower in oxygen tension than optimum for growth of R. equi. This microorganism is reported to be aerobic (Barton and Hughes, 1980).

Future investigations of the incidence of R. equi in fecal or environmental samples probably need not utilize all the media described in this study. Adequate numbers of fecal isolates could have been recovered using direct plating of M3T and both TANP schemes (TNA4, TNA7, TM4, TM7) exclusively. The system including Dex, B64, B67, B124, and B127 could have been reduced to B124 for environmental samples and used in conjunction with the same limited scheme described above for fecal specimens with little change in R.

equi recovery rate.

The most valuable characteristics for identification of R. equi were colony morphology on selective media and blood agar, Gram stain and the CAMP ("equi factor" [Prescott et al., 1982]) reaction. Reactions in triple sugar iron agar, oxidation or fermentation of glucose, hydrolysis of urea and nitrate reduction were also useful.

Prescott et al. (1982) stated that the test for "equi factor" (CAMP test) was more specific for identification of R. equi than other tests. All isolates in both this study and in his study were positive for this test.

Results of the nitrate and urease reactions were read following one week of incubation. Positive results were obtained on 89.1 % and 100 % of the isolates respectively. This compared favorably with the results of Mutimer and Woolcock (1981) who determined that 88 % (nitrate) and 95 % (urease) of 100 cultures were positive for these tests.

One strain which was recovered from a soil sample on the Control Farm was nitrate and urea negative and esculin positive. This isolate compared favorably to an isolate reported by Prescott et al. (1982) which had the same reactions except that he did not report the nitrate reaction. This isolate was CAMP positive also, but in this thesis, was not considered as an R. equi.

Prescott (1981a) extracted antigen for gel diffusion studies by adding saline to living cells and incubating the

suspension without shaking at 37 C. In his hands, antigen prepared in this manner had only faint, adsorbable cross-reacting precipitin lines when reacted with antisera prepared by his method. Antigen prepared in this study by his method failed to react with any of the antisera from early bleedings or with some of the antisera which he donated. This failure led to the development of an alternative method for antigen production. This method was similar to that used by Carter and Hylton (1974) and referred to as "gyrated" antigens. The thought was that increased agitation by shaking in a 37 C water bath would free more capsular material into solution and produce a more concentrated preparation. These antigen preparations did react with antisera from early bleedings, but both homologous and heterologous precipitin bands were observed.

Multiple cross-reactions among sera were a persistent problem during serotyping attempts. Cross-reactions of much less magnitude have plagued other workers. Dimock and Edwards (1931) observed cross-reactions between various strains of R. equi in low dilutions of their agglutination tests. When they conducted precipitin tests, cross-reactions were not evident. Nakazawa (1980) prepared antigen by two different methods from nine strains of R. equi from various sources. Antigens prepared by both methods were tested in AGID against sera from four foals which were infected with R. equi. An antigen which he

referred to as sonicated produced multiple precipitin bands with one of the sera but none with the other three. However, an antigen which was precipitated and dialyzed produced precipitin bands with all four. From the information presented, it appeared that all four foals were infected with the same serotype, that strong cross-reactions occurred between serotypes or that this antigen contained the species specific antigen and not the serotype specific antigens.

Prescott (1981a) also noted faint cross-reacting bands of precipitation in AGID tests. Serotypes 4 and 6 antisera cross-reacted with serotype 2 antigen. Unlike the strong cross-reactions observed in this study, he reported that the homologous reaction was much stronger and distinctive than the cross-reaction. When he adsorbed either cross-reacting serum with serotype 2 cells, the faint cross-reacting precipitin band disappeared, leaving only the strong homologous band.

When it was realized that antigens of each serotype prepared by the alternative method cross-reacted with antiserum prepared against each of the serotypes and that multiple adsorptions of antisera would be necessary, counterimmunoelectrophoresis was selected as the method for additional serotyping. This method was more convenient since it required only approximately 1 hour to complete rather than the 48-72 hours for AGID. This quicker method

allowed interpretation of results sooner, so that additional adsorptions could be done if required.

Although results between AGID and CIEP cannot be directly compared because of the greater sensitivity of CIEP, attempts to adsorb antisera with cells of various serotypes were disappointing. In only one instance was an antiserum able to be completely adsorbed to remove heterologous precipitin bands. This was with anti-serotype 1 and then only after it had been adsorbed 2 times with serotype 2 cells, 4 times with serotype 4 cells and 2 times with serotype 7 cells. In all other instances multiple adsorptions of antisera of other serotypes with cells of various serotypes failed to remove the heterologous precipitin bands. When some antisera were adsorbed with heterologous cells, the homologous reaction was removed or reduced but heterologous reactions remained (see Table 16). When it was realized that adsorption of antisera to make it serotype specific was not likely to be accomplished, additional attempts to serotype the isolates were discarded.

Possibly the reason why these antisera were less specific than those of Prescott (1981a) is because a different immunization protocol was used. It is probable that the route of inoculation ultimately led to the formation of antibodies to species specific antigens and thus multiple cross-reactions (Dimock and Edwards, 1931 and Woodroffe, 1958). Smith and Robinson (1981), Prescott

(1981a) and Bruner and Edwards (1941) obtained satisfactory typing antisera in rabbits using multiple intravenous inoculations which were sometimes accompanied by subcutaneous inoculations.

When immunization is attempted with whole cells of any species of bacteria, the immunization protocol utilized is likely to determine which antigenic determinants to which the animal will respond. In this study, subcutaneous and intramuscular inoculations of living cells emulsified in Freund's Incomplete adjuvant were used as the primary stimulus. Freund's Incomplete adjuvant was used rather than Freund's Complete adjuvant because of the reported common cell wall components between mycobacteria and R. equi (Gordon, 1966; McKenzie et al., 1981 and McKenzie and Ward, 1981). Additional inoculations were living cells injected by the intramuscular route. Apparently these stimuli were more efficient in promoting a response to antigenic determinants which were less well recognized by intravenous injections.

Considering that as many as 12 different precipitin bands developed between the wells in CIEP gels, it is evident that many antigens are present in this type of preparation and consequently on the surface of whole R. equi cells. Attempts to produce typing antisera with the plethora of antigens likely to be present on many if not all cells of a specific species of bacteria may result in an undesirable

requirement for a specific immunization protocol for expression of a specific antigen. When such a procedure is employed, which antigen/s are expressed by antibody production and which are repressed is dependent upon the animal and not the antigen. For example when Prescott (1981a) produced typing antisera to the 7 types of R. equi, an antigen of entirely different chemical composition may have been detected for each serotype. Such serotyping schemes may not truly reflect serotypes, but merely an antigen which is present to a greater extent on one strain of a bacterium and less on another. Future studies of the serotypes of this microorganism should attempt to purify to homogeneity different chemical substances present on the surface of cells and determine their relationship to each other.

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APPENDIX

TABLE 1. Horses Sampled for *Rhodococcus equi* in Feces

<u>CULTURE RESULT</u>	<u>IDENTIFICATION NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY OF FOALS</u>
Negative	H-1	10 years	Quarter Horse	Female	Hay and pelleted ration	None
Negative	H-2	6 years	Thoroughbred	Female	Hay and pelleted ration	None
Negative	H-3	3 weeks	Thoroughbred	Female	Nursing	None
Negative	H-4	2.5 months	Quarter Horse	Female	Nursing	None
Negative	H-5	3 years	Quarter Horse	Female	Hay and pelleted ration	None
Negative	H-6	3 weeks	Quarter Horse	Female	Nursing	None
Positive	H-7	9 years	Quarter Horse	Female	Hay and pelleted ration	None
Negative	H-8	6 years	Quarter Horse	Female	Hay and pelleted ration	None
Positive	H-9	9 years	Thoroughbred	Female	Hay and pelleted ration	None
Negative	H-10	11 years	Thoroughbred	Male	Hay and pelleted ration	None
Negative	H-11	6 years	Thoroughbred	Male	Hay and pelleted ration	None
Positive	H-12	1 year	Thoroughbred	Female	Hay and pelleted ration	None
Positive	H-13	1 year	Quarter Horse	Female	Hay and pelleted ration	None

TABLE 1 (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY</u> <u>OF FOALS</u>
Negative	H-14	1 year	Quarter Horse	Female	Hay and pelleted ration	None
Negative	H-15	1 year	Quarter Horse	Female	Hay and pelleted ration	None
Negative	H-16	1 year	Quarter Horse	Female	Hay and pelleted ration	None
Positive	H-17	1 year	Quarter Horse	Male(c)	Hay and pelleted ration	None
Negative	H-18	1 year	Quarter Horse	Female	Hay and pelleted ration	None
Negative	H-19	1 year	Quarter Horse	Female	Hay and pelleted ration	None
Positive	H-20	7 years	Quarter Horse	Female	Pasture and occasional grain	None
Negative	H-21	1.5 months	Quarter Horse	Male	Nursing	None
Negative	H-22	11 years	Quarter Horse	Female	Pasture and occasional grain	None
Negative	H-23	2 months	Quarter Horse	Female	Nursing	None
Positive	H-24	8 years	Thoroughbred	Female	Pasture and occasional grain	None
Negative	H-25	1 month	Thoroughbred	Female	Nursing	None
Positive	H-26	12 years	Thoroughbred	Female	Pasture and occasional grain	None

TABLE 1 (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY</u> <u>OF FOALS</u>
Positive	K-0A	2 months	Arabian (7/8)	Male	Nursing	Died prior to second sampling, confirmed <u>R. equi</u> infection
Negative	K-1B	1 year	Arabian	Female	Oats and mixed ration	Half sister of K-27A, B
Negative	K-2B	1 year	Arabian	Female	Pasture and occasional grain	None
Negative	K-3B	5 years	Arabian	Female	Hay one week, previously on pasture	None
Positive	K-4B	3 years	Arabian	Male	Hay and grain	None
Negative	K-5B	3 years	Arabian	Male	Hay and grain	None
Negative	K-6B	3 years	Arabian	Male	Hay and grain	None
Negative	K-7B	3 years	Arabian	Female	Hay and grain	None
Negative	K-8B	2 years	Arabian	Male(c)	Pasture, then hay and grain one day	Half brother of K-27A, B
Negative	K-9B	1 year	Arabian	Male	Dry lot, then pasture last ten days	None

TABLE I (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY</u> <u>OF FOALS</u>
Negative	K-10B	6 years	Arabian	Female	Hay and grain	History unknown, outside mare for breeding
Negative	K-11B	12 years	Arabian	Female	Pasture, then hay and grain one day	None
Negative	K-12A	10 years	Arabian	Female	Pasture and occasional grain	Dam of K-1B, K-8B, and K-27A, B
Negative	K-12B	10 years	Arabian	Female	Pasture and occasional grain	Dam of K-1B, K-8B, and K-27A, B
Negative	K-13A	13 years	Arabian	Female	Pasture and occasional grain	None
Negative	K-13A	13 years	Arabian	Female	Pasture and occasional grain	None
Negative	K-14A	6 years	Arabian	Female	Pasture and occasional grain	Dam of foal K-0A, which died of <u>R. equi</u> infection
Negative	K-14B	6 years	Arabian	Female	Pasture and occasional grain	Dam of foal K-0A, which died of <u>R. equi</u> infection
Positive	K-15B	6 years	Arabian	Male	Pasture and occasional grain	None

TABLE I (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY</u> <u>OF FOALS</u>
Negative	K-16B	3 years	Arabian	Female	Pasture and occasional grain	None
Negative	K-17B	3 years	Arabian	Female	Pasture and occasional grain	None
Negative	K-18B	3 years	Arabian	Female	Pasture and occasional grain	None
Negative	K-19B	2 years	Arabian	Female	Pasture and occasional grain	None
Negative	K-20B	12 years	Arabian	Male(c)	Pasture and occasional grain	None
Negative	K-21B	2 years	Arabian	Female	Pasture and occasional grain	None
Negative	K-22B	10 years	Arabian	Female	Pasture and occasional grain	None
Negative	K-23B	10 years	Arabian	Male	Pasture and occasional grain	None
Negative	K-24A	21 years	Arabian (3/4)	Female	Pasture and occasional grain	None
Negative	K-24B	21 years	Arabian (3/4)	Female	Pasture and occasional grain	None

TABLE 1 (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY</u> <u>OF FOALS</u>
Negative	K-25B	6 weeks	Arabian	Male	Nursing	None
Negative	K-26A	2 months	Arabian	Male	Nursing	None
Negative	K-26B	4 months	Arabian	Male	Nursing	None
Positive	K-27A	1 month	Arabian	Female	Nursing	Persistent diarrhea diagnosed as <u>R. equi</u> infection
Negative	K-27B	3 months	Arabian	Female	Nursing	Previous diarrhea diagnosed as <u>R. equi</u> infection
Negative	D-1	12 years	Paint	Male(c)	Hay and grain	None
Negative	D-2	11 years	Paint	Female	Hay and grain	None
Negative	D-3	10 months	Paint	Male	Hay and grain	None
Negative	D-4	2 months	Paint	Female	Hay and grain	None
Negative	D-5	5 years	Quarter Horse	Female	Hay and grain	None
Negative	D-6	6 months	Paint	Female	Hay and grain	None
Negative	D-7	6 months	Paint	Male	Hay and grain	None

TABLE I (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY</u> <u>OF FOALS</u>
Positive	D-8	5 years	Quarter Horse	Female	Pasture	Foal died when 10 days old of undiagnosed intestinal infection
Positive	D-9	5 months	Quarter Horse	Female	Not recorded	Fecal sample collected immediately after death due to <u>R. equi</u> pneumonia
Positive	C-1	10 years	Quarter Horse	Female	Pasture and occasional grain	1977-lost a foal to confirmed <u>R. equi</u> infection
Negative	C-2	12 years	Quarter Horse	Male	Hay and grain	1978-foal survived <u>R. equi</u> infection
Positive	C-3	11 years	Quarter Horse	Female	Pasture and occasional grain	Dam of C-11 and C-12
Negative	C-4	4 years	Quarter Horse	Female	Hay and grain	None
Negative	C-5	1 year	Quarter Horse	Female	Hay and grain	Lost foals in 1976 and 1977, cause unknown
						Contracted confirmed <u>R. equi</u> as a foal
						None

TABLE 1 (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY</u> <u>OF FOALS</u>
Negative	C-6	4 years	Quarter Horse	Male	Hay and grain	None
Positive	C-7	2 years	Quarter Horse	Female	Pasture and occasional grain	Daughter of C-3
Negative	C-8	3 years	Quarter Horse	Female	Hay and grain	None
Positive	C-9	9 years	Quarter Horse	Female	Pasture and occasional grain	None
Negative	C-10	2 years	Quarter Horse	Male	Hay and grain	None
Positive	C-11	4 years	Quarter Horse	Female	Pasture and occasional grain	Contracted confirmed R. equi as a foal Daughter of C-1
Positive	C-12	5 years	Quarter Horse	Female	Pasture and occasional grain	Half sister of C-11
Positive	C-13	32 years	Quarter Horse	Female	Pasture and occasional grain	None
Positive	C-14	20 years	Quarter Horse	Female	Pasture and occasional grain	None

TABLE I (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY</u> <u>OF FOALS</u>
Positive	C-15	10 years	Quarter Horse	Female	Pasture and occasional grain	None
Positive	C-16	18 years	Quarter Horse	Female	Pasture and occasional grain	None
Positive	C-17	15 years	Quarter Horse	Female	Pasture and occasional grain	Dam of C-4
Positive	C-18	7 years	Quarter Horse	Female	Pasture and occasional grain	None
Negative	C-19	8 years	Quarter Horse	Female	Pasture and occasional grain	None
Positive	C-20	18 years	Quarter Horse	Female	Pasture and occasional grain	None
Negative	C-21	9 years	Quarter Horse	Female	Pasture and occasional grain	None
Positive	C-22	6 years	Quarter Horse	Female	Pasture and occasional grain	Half sister of C-4
Positive	C-23	1 year	Quarter Horse	Female	Hay and grain	None
Negative	C-24	13 years	Quarter Horse	Female	Pasture and occasional grain	None

TABLE I (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY</u> <u>OF FOALS</u>
Positive	001	Adult	Quarter Horse	Female	Hay and grain	Unknown
Positive	002	Adult	Arabian	Male	Hay and grain	Unknown
Positive	003	7 years	Quarter Horse	Female	Hay and grain	Unknown
Positive	004	12 years	Clydesdale	Female	Hay and grain	Unknown
Positive	005	15 years	Quarter Horse	Male(c)	Hay and grain	Unknown
Positive	006	6 years	Quarter Horse	Female	Hay and grain	Unknown
Negative	007	3 years	Quarter Horse	Female	Hay and grain	Unknown
Negative	008	13 years	Thoroughbred	Female	Hay and grain	Unknown
Positive	009	1 month	Thoroughbred	Male	Nursing	None
Positive	010	9 years	Thoroughbred	Female	Hay and grain	Unknown
Positive	011	7 years	Thoroughbred	Male	Hay and grain	Unknown
Positive	012	1 year	Thoroughbred	Male(c)	Hay and grain	Unknown
Positive	013	2 years	Quarter Horse	Female	Hay and grain	Unknown
Positive	014	14 years	Quarter Horse	Male	Hay and grain	Unknown
Positive	015	4 months	Thoroughbred	Female	Weaning, hay and grain	Unknown

TABLE 1 (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>AGE</u>	<u>BREED</u>	<u>SEX</u>	<u>RATION</u>	<u>HISTORY</u> <u>OF FOALS</u>
Negative	016	7 months	Appaloosa	Female	Hay and grain	Unknown
Negative	017	5 months	Quarter Horse	Female	Hay and grain	Unknown
Positive	018	4 years	Quarter Horse	Male(c)	Hay and grain	Unknown
Positive	019	5 years	Quarter Horse	Male	Hay and grain	Unknown
Positive	020	3 years	Thoroughbred	Male(c)	Hay and grain	Unknown
Positive	021	Unknown	Unknown	Unknown	Hay and grain	Unknown

TABLE II. Horses Sampled for *Rhodococcus* equi in Feces

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>DATE</u> <u>SAMPLED</u>	<u>KANSAS</u> <u>LOCATION</u>	<u>TIME</u> <u>AT FARM</u>	<u>TREATMENT</u>	<u>HEALTH</u> <u>STATUS</u>
Negative	H-1	6/18/82	Alta Vista	5 years	None	Normal
Negative	H-2	6/18/82	Alta Vista	4 years	None	Normal
Negative	H-3	6/18/82	Alta Vista	3 weeks	None	Normal
Negative	H-4	6/18/82	Alta Vista	2 weeks	None	Normal
Negative	H-5	6/18/82	Alta Vista	3 years	None	Normal
Negative	H-6	6/18/82	Alta Vista	3 weeks	None	Normal
Positive	H-7	6/18/82	Alta Vista	3 years	None	Normal
Negative	H-8	6/18/82	Alta Vista	5 years	None	Normal
Positive	H-9	6/18/82	Alta Vista	2 years	None	Normal
Negative	H-10	6/18/82	Alta Vista	2 years	None	Normal
Negative	H-11	6/18/82	Alta Vista	2 years	None	Normal
Positive	H-12	6/18/82	Alta Vista	1 year	None	Normal
Positive	H-13	6/18/82	Alta Vista	1 year	None	Normal
Negative	H-14	6/18/82	Alta Vista	1 year	None	Normal
Negative	H-15	6/18/82	Alta Vista	1 year	None	Normal

TABLE II (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>DATE</u> <u>SAMPLED</u>	<u>KINGS</u> <u>LOCATION</u>	<u>TIME</u> <u>AT FARM</u>	<u>TREATMENT</u>	<u>HEALTH</u> <u>STATUS</u>
Negative	H-16	6/18/82	Alta Vista	1 year	None	Normal
Positive	H-17	6/18/82	Alta Vista	1 year	None	Normal
Negative	H-18	6/18/82	Alta Vista	1 year	None	Normal
Negative	H-19	6/18/82	Alta Vista	1 year	None	Normal
Positive	H-20	6/18/82	Alta Vista	2 years prior to recent absence, just arrived	None	Normal
Negative	H-21	6/18/82	Alta Vista	Just arrived	None	Normal
Negative	H-22	6/18/82	Alta Vista	1.5 years prior to recent absence, just arrived	None	Normal
Negative	H-23	6/18/82	Alta Vista	Just arrived	None	Normal
Positive	H-24	6/18/82	Alta Vista	5 years prior to recent absence, Just arrived	None	Normal
Negative	H-25	6/18/82	Alta Vista	Just arrived	None	Normal
Positive	H-26	6/18/82	Alta Vista	5 years	None	Normal

TABLE II (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>DATE</u> <u>SAMPLED</u>	<u>KANSAS</u> <u>LOCATION</u>	<u>TIME</u> <u>AT FARM</u>	<u>TREATMENT</u>	<u>HEALTH</u> <u>STATUS</u>
Positive	K-0A	5/27/82	Valley Falls	2 months	Not recorded	Loose feces when tested
Negative	K-1B	7/7/82	Valley Falls	1 year	None	Normal
Negative	K-2B	7/7/82	Valley Falls	1 year	None	Normal
Negative	K-3B	7/7/82	Valley Falls	5 years	None	Normal
Positive	K-4B	7/7/82	Valley Falls	2 years	None	Normal
Negative	K-5B	7/7/82	Valley Falls	3 years	None	Normal
Negative	K-6B	7/7/82	Valley Falls	10 months	None	Normal
Negative	K-7B	7/7/82	Valley Falls	Born here, sold Recently returned	None	Normal
Negative	K-8B	7/7/82	Valley Falls	2 years	None	Normal
Negative	K-9B	7/7/82	Valley Falls	1 year	None	Normal
Negative	K-10B	7/7/82	Valley Falls	1 month	None	Normal
Negative	K-11B	7/7/82	Valley Falls	9 years	None	Normal
Negative	K-12A	5/27/82	Valley Falls	3 years	None	Normal
Negative	K-12B	7/7/82	Valley Falls	3 years	None	Normal

TABLE II (continued)

<u>CULTURE RESULT</u>	<u>IDENTIFICATION NUMBER</u>	<u>DATE SAMPLED</u>	<u>KANSAS LOCATION</u>	<u>TIME AT FARM</u>	<u>TREATMENT</u>	<u>HEALTH STATUS</u>
Negative	K-13A	5/27/82	Valley Falls	3 years	None	Normal
Negative	K-13B	7/7/82	Valley Falls	3 years	None	Normal
Negative	K-14A	5/27/82	Valley Falls	3 years	None	Normal
Negative	K-14B	7/7/82	Valley Falls	3 years	None	Normal
Positive	K-15B	7/7/82	Valley Falls	11 months	None	Normal
Negative	K-16B	7/7/82	Valley Falls	2 years	None	Normal
Negative	K-17B	7/7/82	Valley Falls	3 years	None	Normal
Negative	K-18B	7/7/82	Valley Falls	2 months	None	Normal
Negative	K-19B	7/7/82	Valley Falls	2 years	None	Normal
Negative	K-20B	7/7/82	Valley Falls	12 years	None	Normal
Negative	K-21B	7/7/82	Valley Falls	10 months	None	Normal
Negative	K-22B	7/7/82	Valley Falls	7 years prior to 1 year absence, Now here 2 weeks	None	Normal
Negative	K-23B	7/7/82	Valley Falls	8 years	None	Normal
Negative	K-24A	5/27/82	Valley Falls	10 years	None	Normal

TABLE II (continued)

<u>CULTURE RESULT</u>	<u>IDENTIFICATION NUMBER</u>	<u>DATE SAMPLED</u>	<u>KANSAS LOCATION</u>	<u>TIME AT FARM</u>	<u>TREATMENT</u>	<u>HEALTH STATUS</u>
Negative	K-24B	7/7/82	Valley Falls	10 years	None	Normal
Negative	K-25B	7/7/82	Valley Falls	2 weeks	Not recorded	Normal
Negative	K-26A	5/27/82	Valley Falls	2 months	Not recorded	Recovered Group F <u>Salmonella sp.</u>
Negative	K-26B	7/7/82	Valley Falls	4 months	Not recorded	Recovered Group F <u>Salmonella sp.</u> From this fecal on 1st sampling
Positive	K-27A	5/27/82	Valley Falls	1 month	Not recorded	Loose feces. No <u>Salmonella sp.</u> isolated
Negative	K-27B	7/7/82	Valley Falls	3 months	None currently. Gentocin earlier for diarrhea	Persistent diarrhea in spring <u>R. equi</u> culture- positive
Negative	D-1	9/30/82	Chapman	3 years	None	Normal
Negative	D-2	9/30/82	Chapman	1 year	None	Normal
Negative	D-3	9/30/82	Chapman	10 months	None	Respiratory problem
Negative	D-4	9/30/82	Chapman	2 months	None	Normal

TABLE II (continued)

<u>CULTURE RESULT</u>	<u>IDENTIFICATION NUMBER</u>	<u>DATE SAMPLED</u>	<u>KANSAS LOCATION</u>	<u>TIME AT FARM</u>	<u>TREATMENT</u>	<u>HEALTH STATUS</u>
Negative	D-5	9/30/82	Chapman	3 years	None	Normal
Negative	D-6	9/30/82	Chapman	6 months	Not recorded	Coughing and serous nasal discharge
Negative	D-7	9/30/82	Chapman	6 months	Tribissen	Coughing and serous nasal discharge
Positive	D-8	9/30/82	Chapman	3 years	None	Normal
Positive	D-9	9/25/82	Manhattan	5 months	Antimicrobials	Confirmed R. equi infection
Positive	C-1	10/2/82	Tonganoxie	6 years	None	Normal
Negative	C-2	10/2/82	Tonganoxie	10 years	None	Normal
Positive	C-3	10/2/82	Tonganoxie	11 years	None	Normal
Negative	C-4	10/2/82	Tonganoxie	4 years	None	Normal
Negative	C-5	10/2/82	Tonganoxie	1 year	None	Normal
Negative	C-6	10/2/82	Tonganoxie	3 years	None	Normal
Positive	C-7	10/2/82	Tonganoxie	2 years	None	Normal
Negative	C-8	10/2/82	Tonganoxie	3 years	None	Normal
Positive	C-9	10/2/82	Tonganoxie	9 years	None	Normal

TABLE II (continued)

<u>CULTURE RESULT</u>	<u>IDENTIFICATION NUMBER</u>	<u>DATE SAMPLED</u>	<u>KANSAS LOCATION</u>	<u>TIME AT FARM</u>	<u>TREATMENT</u>	<u>HEALTH STATUS</u>
Negative	C-10	10/2/82	Tonganoxie	2 years	None	Normal
Positive	C-11	10/2/82	Tonganoxie	4 years	None	Normal
Positive	C-12	10/2/82	Tonganoxie	5 years	None	Normal
Positive	C-13	10/2/82	Tonganoxie	10 years	None	Normal
Positive	C-14	10/2/82	Tonganoxie	11 years	None	Normal
Positive	C-15	10/2/82	Tonganoxie	5 years	None	Normal
Positive	C-16	10/2/82	Tonganoxie	5 years	None	Normal
Positive	C-17	10/2/82	Tonganoxie	6 years	None	Normal
Positive	C-18	10/2/82	Tonganoxie	4 years	None	Normal
Negative	C-19	10/2/82	Tonganoxie	1 year	None	Normal
Positive	C-20	10/2/82	Tonganoxie	6 years	None	Normal
Negative	C-21	10/2/82	Tonganoxie	9 years	None	Normal
Positive	C-22	10/2/82	Tonganoxie	6 years	None	Normal
Positive	C-23	10/2/82	Tonganoxie	1 year	None	Normal
Negative	C-24	10/2/82	Tonganoxie	12 years	None	Normal

TABLE II (continued)

<u>CULTURE RESULT</u>	<u>IDENTIFICATION NUMBER</u>	<u>DATE SAMPLED</u>	<u>KANSAS LOCATION</u>	<u>TIME AT PAIN</u>	<u>TREATMENT</u>	<u>HEALTH STATUS</u>
Positive	001	4/12/82	Manhattan	63 days	None recorded	Normal
Positive	002	4/12/82	Manhattan	38 days	None recorded	Normal
Positive	003	4/12/82	Manhattan	17 days	None recorded	Normal
Positive	004	4/12/82	Manhattan	5 days	None recorded	Infertility, endometritis
Positive	005	4/12/82	Manhattan	3 days	None recorded	Sesamoid chips
Positive	006	4/12/82	Manhattan	2 days	None recorded	Reproductive exam. No heat. Won't tolerate stallion
Negative	007	6/17/82	Manhattan	6 days	None	Normal (to be bred)
Negative	008	6/17/82	Manhattan	13 days	None	Normal (to be bred)
Positive	009	6/17/82	Manhattan	13 days	None	Normal
Positive	010	6/17/82	Manhattan	2 days	None	Normal (to be bred)
Positive	011	6/17/82	Manhattan	14 days	None recorded	Hook problem
Positive	012	9/20/82	Manhattan	28 days	None recorded	Wobbler
Positive	013	9/20/82	Manhattan	1 day	Tribriksen, phenylbutasone left hock	Septic arthritis left hock

TABLE II (continued)

<u>CULTURE</u> <u>RESULT</u>	<u>IDENTIFICATION</u> <u>NUMBER</u>	<u>DATE</u> <u>SAMPLED</u>	<u>KANSAS</u> <u>LOCATION</u>	<u>TIME</u> <u>AT FARM</u>	<u>TREATMENT</u>	<u>HEALTH</u> <u>STATUS</u>
Positive	014	9/20/82	Manhattan	6 days	Procaine penicillin, phenylbutasone	Bladder stone
Positive	015	9/20/82	Manhattan	20 days	None recorded	Normal (weaning)
Negative	016	9/20/82	Manhattan	7 days	None recorded	Wobbler
Negative	017	9/21/82	Manhattan	6 days	Phenylbutasone	Head tilt from flipping over backward
Positive	018	9/21/82	Manhattan	6 days	Procaine penicillin, phenylbutasone, DMSO	Colic
Positive	019	9/21/82	Manhattan	1 day	Phenylbutasone	Laminitis
Positive	020	9/21/82	Manhattan	8 days	Procaine penicillin, phenylbutasone	Eastern laceration
Positive	021	9/21/82	Manhattan	2 days	Unknown	Bladder stone

EPIDEMIOLOGY OF RHODOCOCCLUS (CORYNEBACTERIUM) EQUI IN FECAL
AND ENVIRONMENTAL SAMPLES FROM KANSAS HORSES AND LOCATIONS

by

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An Abstract of

A MASTER'S THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Department of Laboratory Medicine

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1986

Abstract

The habitat of Rhodococcus equi and the source of infection for foals has been an area of debate. The purpose of this study was to compare the incidence of R. equi in horse feces and in the environment from locations with various histories of R. equi pneumonia. The organism was recovered from heavily contaminated fecal and environmental samples using selective media.

Four farms were selected from which to collect specimens. The Control Farm had no clinical history of R. equi foal pneumonia. The farms designated as Infected Farm I and Infected Farm II experienced R. equi foal pneumonia that season. Three and four years prior to sampling, foals died of R. equi pneumonia on the Previously Infected Farm, but no infections by this organism had occurred since that time. In addition, fecal samples from 21 horses which were hospitalized at the Kansas State University (KSU) Veterinary Hospital were cultured for R. equi.

Environmental samples were collected at all locations, but recovery rates were generally low. Seven of 47 soil samples (14.9 %) and 2 of 33 cobweb samples (6.1 %) yielded cultures of R. equi. No R. equi was recovered from dust on 12 different ceiling filters above the equine stalls of the KSU Veterinary Hospital.

A total of 114 horses representing 6 different breeds were sampled from the 5 locations. Forty-six (40.4 %) of

these fecal specimens yielded cultures of R. equi. Horses which were at the KSU Veterinary Hospital had a significantly higher ($p = 0.000$) incidence (88.9 %) of R. equi than horses which were on farms (31.2 %). In this study increased incidence of the organism in horse feces on the farm did not correspond with increased incidence of R. equi foal pneumonia.

Attempts to develop adequate capsular serotyping antisera in rabbits using subcutaneous and intramuscular inoculation of live organisms were not successful. Consequently, the relationship between strains of R. equi from foal pneumonias and isolates from the foals' habitats could not be determined.